Medical Library

UNIVERSITY
OF MICHIGAN
OCT 27 1950
MEDICAL
LIBRARY

Vol. 40, Nos. 3, 4 October 1950

PARASITOLOGY

(Founded by G. H. F. Nuttall)

EDITED BY

D. KEILIN

IN CONJUNCTION WITH

A. BISHOP
F. J. BROWN
P. A. BUXTON
C. DOBELL
E. HINDLE
C. A. HOARE
G. LAPAGE
K. M. SMITH
P. TATE

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 51 MADISON AVENUE

Thirty-seven Shillings net

Issued 10 *October* 1950

A
Naturalist in the
Gran Chaco

BY

SIR JOHN GRAHAM KERR

24 plates 2 maps

21s. net

Cambridge
University Press

BRAND **MICHROME** BRAND

STAINS & REAGENTS FOR MICROSCOPY

Azocarmine B & G	Janus Red
Azur I & II	Jenner Stain
Brilliant Cresyl Blue	Leishman Stain
Cedarwood Oil	May-Grunwald Stain
Evans Blue	Nile Blue Sulphate
Giemsa Stain	Pontamine Sky Blue
Janus Blue	Rosolic Acid
Janus Green	Sudan Blue

Water Soluble Indicators

Stains in tablet form Etc. Etc.

Microscope Slides, Coverslips
Water Testing Tablets

Price Lists on application

Now ready: Microscopic Staining Techniques
No. 1 (24 pp.) 1s. 6d. No. 2 (62 pp.) 3s. 6d.

●

EDWARD GURR LTD.
108 WATERFORD ROAD
WALHAM GREEN, LONDON, S.W.6
Tel.: Renown 2011 Cables: Gurr, Renown 2011, London
UNIFORM HIGH QUALITY, RELIABILITY AND IMMEDIATE DELIVERY

The TREMATODA

BEN DAWES

A detailed study of the trematode parasites of vertebrate animals such as the advanced student of zoology dissects in the laboratory, intended to give zoologists and parasitologists a more useful account of the Trematoda than can be found in existing works. The author is Lecturer in Zoology at King's College, London.

52s. 6d. net

CAMBRIDGE UNIVERSITY PRESS

VOLUME 40, NOS. 3 & 4 OCTOBER 1950

AN EXPERIMENTAL STUDY OF EAST COAST FEVER IN UGANDA

I. A STUDY OF THE TYPE OF EAST COAST FEVER REACTIONS PRODUCED WHEN THE NUMBER OF INFECTED TICKS IS CONTROLLED

BY S. G. WILSON, *Veterinary Research Laboratory, Entebbe, Uganda*

(With 24 Figures in the Text)

CONTENTS

	PAGE
I. Introduction	195
II. Material and Methods	195
III. Experimental results	196
(1) East Coast fever reactions without tick control	196
(2) East Coast fever reactions following partial tick control	196
(3) East Coast fever reactions in calves showing *Theileria mutans* infection	203
(4) East Coast fever reactions when infected ticks are reduced to a minimum	204
(5) East Coast fever reactions following partial tick control by feeding benzene hexachloride to calves	204
(6) East Coast fever reactions when definite numbers of infected ticks were fed on susceptible animals	204
Summary	208
References	209

I. INTRODUCTION

A disease in calves, known locally as 'Amakebe' and caused by *Theileria parva* (East Coast fever), has been recognized in Uganda for many years (Bruce, 1910). One of the most striking features of this disease was the variable mortality which occurred from year to year and from place to place. The factors governing this variability have never been fully elucidated. Mettam (1933) reported that when fourteen calves were exposed to the infestation of infected ticks in an experimental paddock at Entebbe, 'the nature of the reactions varied greatly'. As a result, he assumed that the 'individual resistance or susceptibility' was the 'most important factor in pathogenesis of the disease in indigenous cattle'. Mettam & Carmichael (1936) estimated that mortality under field conditions in Uganda varied from 10 to 40%, and considered that indigenous cattle, as the result of exposure to infected ticks over many centuries, had developed a variable degree of resistance which in some cases had become pronounced.

Hornby (1934), in a survey of the East Coast fever position in Tanganyika, admitted that it was not known why 50% of the young calves in enzootic areas recovered or whether it was due to immune bodies received from mother's milk, or was a function of age or if a genetical factor was concerned.

Fotheringham & Lewis (1937) report mild atypical reactions following artificial infections. In a later study designed to show if the disease could be modified by 'the route of infection and by the degree of tick infestation', Lewis & Fotheringham (1941) concluded that, although the mild form of the disease seems associated at times with a light tick infestation, it was proved that a few infected ticks also transmit a fatal East Coast fever infection. In one particular experiment (p. 257), when mild reactions occurred, the authors were unable to decide whether the atypical reactions were due to mild tick infections or to the better conditions of the animals.

From a study of the epidemiology of the disease in Nyasaland (Wilson, 1944, 1945, 1946) it was shown that acute fatal *T. parva* infections were most prevalent each year during the season when *Rhipicephalus appendiculatus* ticks were most abundant. The results suggested that the number of ticks infecting an animal was a factor which influenced the subsequent course of the disease. The work described in this paper deals largely with investigations into this aspect of the problem.

II. MATERIAL AND METHODS

Certain factors which might influence the course of the East Coast fever reactions have been kept uniform throughout the experiment. All the calves were indigenous Nganda breed, bred on the laboratory farm under almost tick-free conditions, and their morning temperatures had been recorded from birth. Their management and feeding had all been on

very similar lines. The age of the animals on experiment varied, but all had been weaned, and as many of their dams had been reared on the laboratory farm and had no previous history of *Theileria parva* infections it is improbable that any immunity had been transferred from mother to calf. Only one species of tick-vector, *Rhipicephalus appendiculatus*, was used. In Exps. 1–4 the calves were infected with *Theileria parva* by allowing them to graze in a 1-acre paddock infested with infected *Rhipicephalus appendiculatus* ticks. During Exp. 1 the number of infected ticks in the paddock was high, but during the course of the experiments every attempt was made gradually to reduce their numbers by grazing immune animals in the paddock, by killing with D.D.T. sprays or by removing reacting animals early in the course of their reactions, until in Exp. 4 the number was estimated as low. In Exp. 5 the susceptible calves were grazed and housed with an indigenous herd and all were subjected to a variable tick infestation. In Exp. 6, known numbers of infected *R. appendiculatus* nymphs were attached directly on to the ears of the calves by means of ear-bags, attached with elastoplast. The chief conditions therefore which varied throughout the experiments were the age of the animals and the number of infected ticks which engorged. During each experiment the morning temperatures of the calves were recorded, and blood smears were examined daily from the first day of exposure to infection and gland smears were examined daily from the first day of temperature rise.

III. EXPERIMENTAL RESULTS

(1) *East Coast fever reactions without tick control*

Ten young bulls, varying in ages from 13 to 35 months, with no previous history of having been exposed to *Theileria parva*, were grazed in a tick-infested paddock. *Rhipicephalus appendiculatus* was the chief tick species present, and an unknown portion of them was infected with *Theileria parva*. The nature of the East Coast fever reactions which resulted are given in Table 1. Out of ten animals in the experiment, eight died of typical acute reactions from 28 to 40 days after exposure. The incubation period varied from 9 to 14 days, the temperature remaining above 104° F. for 8–11 days and Koch's bodies were found consistently in gland smears for 12–14 days (Fig. 1). Mild reactions occurred in only two cases. The incubation period was 16 and 12 days respectively, Koch's bodies were found in gland smears on 7 and 2 days only, and the temperature remained above 104° F. for 6 and 4 days (Fig. 2). All the bulls were of local Nganda type and in good condition, and the mild reactions did not appear to depend on the age of the animals. The presence of any variation in the individual resistance of the animals can only be assumed. It is, however, reasonable to suppose that in a paddock of this nature where the number of infected ticks was limited and when many (10) animals were exposed, the rate of infestation with infected ticks would vary, and the two recovered animals might well be those on which a small number of infected ticks fed.

(2) *East Coast fever reactions following partial tick control*

(*a*) Six calves, susceptible to East Coast fever, and varying in ages from 5 to 26 months, were grazed from 13 August to 24 September 1946, in the same tick-infested paddock used in the previous experiment. In order to reduce the number of infected ticks likely to engorge, two immune calves were also grazed in this paddock and, since in natural infestations the nymphs and adults of *Rhipicephalus appendiculatus* chiefly attached on the ears of bovines, the ears of two calves, nos. 5583 and 5562, were sprayed once with 5–10 c.c. of a 5 % D.D.T. emulsion on the second day only, and four calves, nos. 5392, 5465, 5467 and 5416, were sprayed on the 1st and 9th days with 30 c.c. of the same emulsion. The details of the daily tick counts are given in another paper (Wilson, 1948*a*). It is sufficient here to note that the average number of ticks on the ears of the group of four calves varied from nil to 1·15 ticks per animal until the 18th day, when they gradually increased to over six ticks, whilst the average number on the ears of the two calves during the same period varied from two to nine ticks per animal. The chief features of the reactions in the six susceptible animals are summarized in Table 2.

The two calves which were lightly sprayed on the 2nd day only reacted strongly. The reaction shown by no. 5583 (Fig. 3) was that of a typically fatal *Theileria parva* infection, with a normal incubation period and a morning temperature consistently above 104° F. for 18 days. Schizonts were present in gland smears for 13 consecutive days. The number of erythrocytes parasitized by piroplasms gradually increased from the 20th day onwards, until 85 % of these cells contained one to three parasites on the 31st day, when death occurred. The reaction of 5562 was less severe (Fig. 4). The incubation period was slightly prolonged, and the temperature was 104° F. or over for only 12 days. Schizonts were found in gland smears intermittently on 6 days only, from the 24th to 33rd day of exposure, and the number of parasitized erythrocytes varied from 4 to 9 %. Recovery was uneventful.

In the group of four calves, where spraying was more thorough, the onset of symptoms was delayed until the 27th–30th day. The reaction in 5392, the oldest heifer in the group, was sufficiently severe to cause death (Fig. 5). In the three young heifers which

Table 1. *East Coast fever reactions in ten susceptible bovines exposed to tick infestations, without tick control*

Bovine and sex	Age (months)	Day after exposure. Temp. above 103° F. with swollen glands	Maximum temp. during reaction	Days on which K.B.B. found in gland smears	Days on which *Theileria* found in blood	Result
5358 ♂	35	16th day	105·1° F. on 19th day	17th–23rd day	17th–28th day	Recovered
5436 ♂	24	12th day	106° F. on 14th day	16th and 17th days	20th–28th day	Recovered
5360 ♂	35	9th day	105·4° F. on 13th day	15th–28th day	16th–28th day	Died 28th day
5442 ♂	22	11th day	105·4° F. on 16th day	14th–33rd day	15th–33rd day	Died 33rd day
5413 ♂	27	10th day	105° F. on 14th day	16th–32nd day	19th–32nd day	Died 32nd day
5439 ♂	24	10th day	104·4° F. on 28th day	24th–27th day and 39th–41st day	22nd–41st day	Died 41st day
5393 ♂	29	13th day	105·6° F. on 22nd day	6th–33rd day	22nd–33rd day	Died 33rd day
5536 ♂	13	14th day	106·2° F. on 17th day	17th–19th day and 24th–36th day	20th–36th day	Died 36th day
5376 ♂	32	13th day	106° F. on 17th day	17th–30th day	20th–30th day	Died 30th day
5471 ♂	18	13th day	105° F. on 14th day	16th–36th day	20th–36th day	Died 36th day

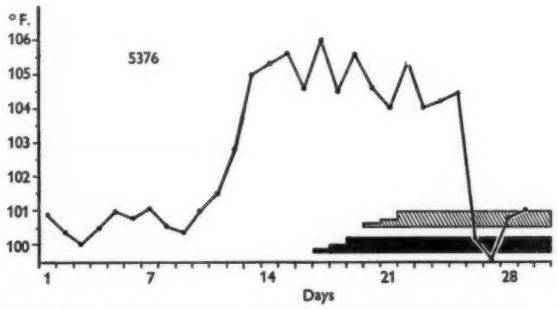


Fig. 1. Calf 5376, an acute fatal East Coast fever reaction similar to that shown by seven other calves in Exp. 1. See key on p. 209.

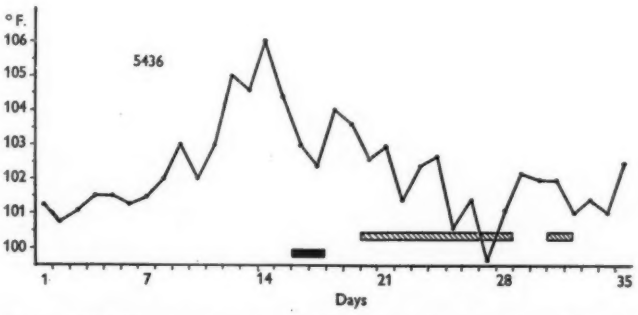


Fig. 2. Calf 5436, a non-fatal type of East Coast fever reaction, also shown by calf 5358 in Exp. 1.

Table 2. *East Coast fever reactions in six susceptible bovines following partial tick control*

Bovine no. and sex	Age (months)	Day after exposure. Temp. above 103° F. with swollen glands	Maximum temp. during reaction	Days on which K.B.B. found in gland smears	Days on which *Theileria* found in blood	Result
5583♂	5	13th day	107° F. on 16th and 17th days	19th–31st day	20th–31st day	Died 31st day
5562♀	8	16th day	106° F. on 21st day	Scanty and intermittent from 24th to 33rd day	Scanty from 19th to 37th day	Recovered
5392♀	26	30th day	105·2° F. on 37th and 39th days	35th–47th day	33rd–47th day	Died 47th day
5465♀	13	27th day	104·6° F. on 32nd day	33rd and 41st days only	32nd–42nd day	Recovered
5467♀	13	28th day	104·8° F. on 32nd day	32nd–34th and 36th–40th day	32nd–46th day	Recovered
5416♀	11	27th day	104·6° F. on 32nd, 104·9° F. on 40th day	33rd and 34th days	32nd–39th day	Recovered

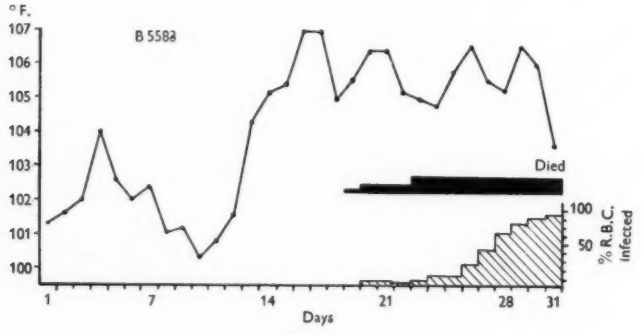


Fig. 3.

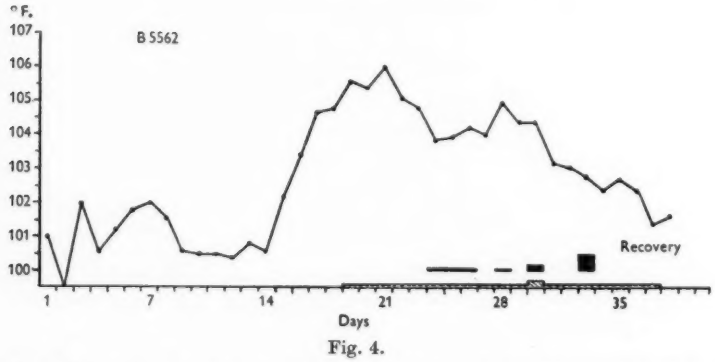


Fig. 4.

Figs. 3, 4. East Coast fever reactions in two calves when there was little tick control. Fig. 3. Calf 5583, an acute fatal case, showing percentage of erythrocytes parasitized with *T. parva*. Fig. 4. An acute non-fatal case with Koch's bodies present intermittently for only 6 days.

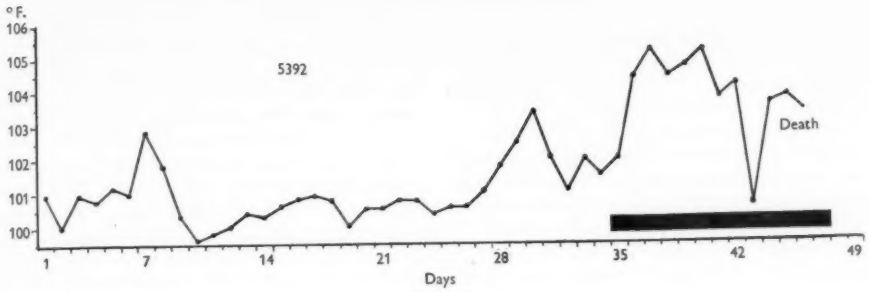


Fig. 5.

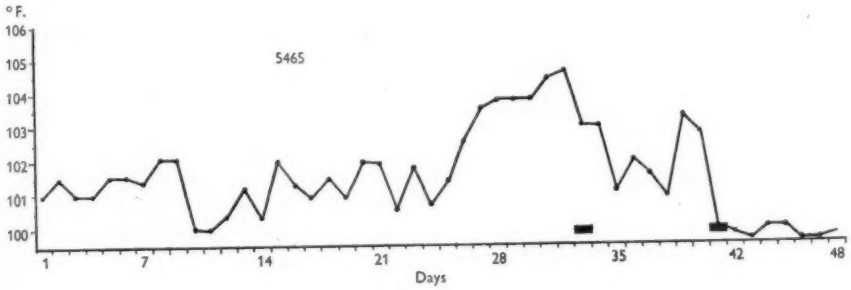


Fig. 6.

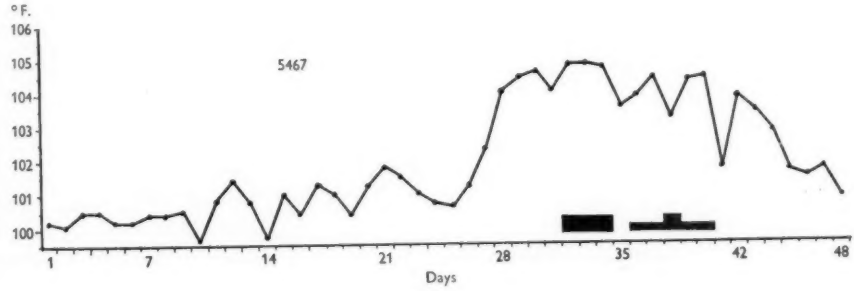


Fig. 7.

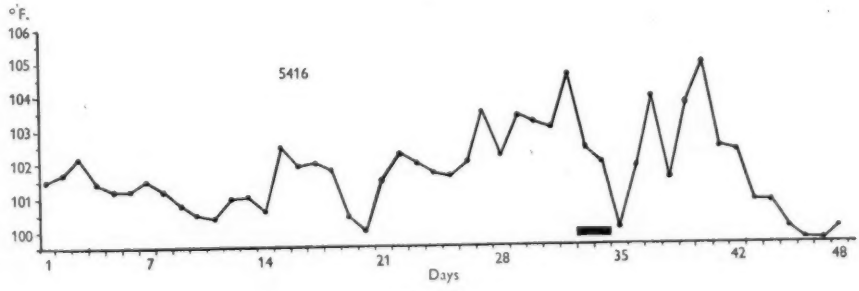


Fig. 8.

Figs. 5–8. East Coast fever reactions in four calves when tick infestation was controlled by ear-sprayings and onset of symptoms was delayed. Fig. 5. Calf 5392, aged 26 months, showed an acute fatal attack. Figs. 6–8. Calves 5465, 5467 and 5416, aged 11–13 months, showed mild attacks with recovery.

recovered, the maximum temperature was always less than 105° F. and only exceeded 104° F. on 2, 8 and 2 days respectively, while schizonts were present in gland smears on 2, 8 and 2 days only (Figs. 6–8).

The results of this experiment showed that in young calves a reduction in the number of infected ticks engorging appeared to favour the development of a non-fatal type of *T. parva* infection. This line of investigation was therefore continued.

(*b*) Nine further susceptible calves were grazed in this paddock from 24 September until reactions had terminated by death or recovery. The ears of three calves were sprayed twice with a D.D.T. solution, three were sprayed with a 'Gammexane' preparation and three were left unsprayed and tick counts were made daily (Wilson, 1948*a*). The reactions in each animal are given in Table 3.

The tick counts were low, as some of the original population had been killed by the D.D.T. spraying in the first experiment and others had engorged and were 'underground' moulting. For this reason no further spraying was done after the 7th day, but even so the tick counts were difficult to assess. An analysis of the counts on the 12th–14th days of

Table 3. *East Coast fever reactions in nine susceptible calves following partial tick control*

Bovine no. and sex	Age (months)	Spray treatment of ears	Day after exposure. Temp. above 103°F. with swollen glands	Max. temp. during reaction	Days on which K.B.B. found in gland smears	Days on which *Theileria* found in blood	Result
5396♀	27	30 c.c. D.D.T.	30th day	105°F. on 34th day	33rd day until death	33rd day until death	Died 46th day of typical E.C.F.
5464♂	16	26 c.c. D.D.T.	31st day	104·4°F. on 38th day	Nil	20th–40th day	Glands swollen 19th–47th day. Recovered
5566♂	9	20 c.c. D.D.T.	33rd day	105°F. on 45th day	40th–42nd day	40th–53rd day	Glands swollen 23rd–60th day. Recovered
5437♀	22	30 c.c. 'Gammexane'	18th and 31st days	105·4°F. on 26th day	Scanty 23rd day, 32nd day until death	33rd day until death	Died 46th day typical E.C.F.
5469♂	17	28 c.c. 'Gammexane'	31st day	104·5°F. on 32nd day	35th day only	27th, 39th, 43rd days	Glands swollen 16th, 48th days. Recovered
5575♂	10	32 c.c. 'Gammexane'	30th day	105°F. on 32nd day	35th day only	Intermittent 35th–42nd day	Glands swollen 23rd–50th day. Recovered
5395♀	27	Control not sprayed	17th day	106°F. on 25th day	24th, 40th, 45th–46th days	Intermittent 27th–45th day	Glands swollen 19th–60th day. Recovered
5478♂	15	Control not sprayed	14th day	106·2°F. on 39th day	33rd–34th, 36th, 38th–44th days	33rd–49th day	Glands swollen 33rd–60th day. Recovered
5573♂	10	Control not sprayed	30th day	103·2°F. on 32nd day	32nd–33rd and 42nd days	Intermittent 33rd–43rd day	Died 45th day

exposure showed that the calves in the D.D.T.-sprayed group carried an average of one adult *Rhipicephalus appendiculatus* and two nymphs each, those of the 'Gammexane'-sprayed group carried two adults and three nymphs, while the unsprayed calves carried 3 adults and over five nymphs each. The individual infestation, however, varied greatly, and the above counts are only given as a rough approximate estimation.

The chief effect of this particular method of controlling ticks was that the first signs of reaction in five of the six treated animals were prolonged until the 30–33rd days, whereas amongst the three unsprayed, two reacted severely on the 14th and 17th days and the third died. The results, however, can probably best be assessed by treating the three groups as a whole, and by regarding the experiment as a study of East Coast fever reactions when susceptible bovines are exposed to a low tick infestation and where mortality is limited to 30 % approximately.

The reactions in the three older animals, one from each group, are shown in Figs. 9–11. Bovine 5396 showed a typical East Coast fever reaction, with death occurring after schizonts had been present in gland smears for 14 consecutive days. Bovine 5437 developed an early reaction on the 18th day with

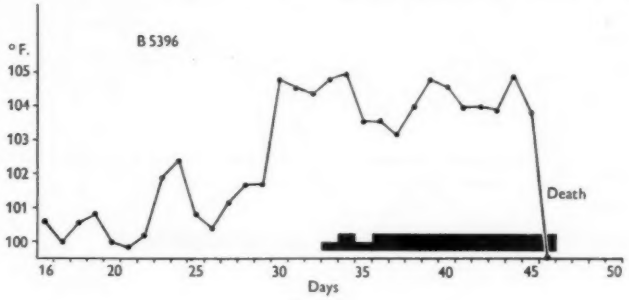


Fig. 9.

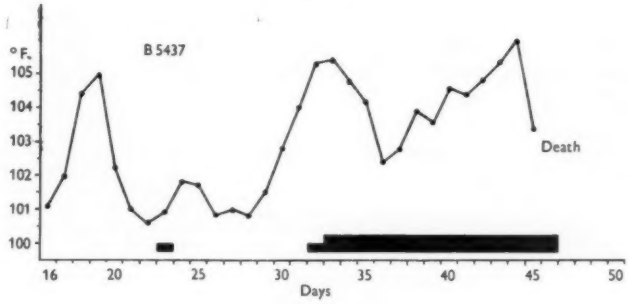


Fig. 10.

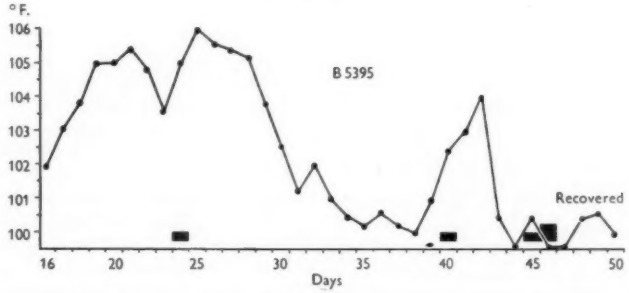


Fig. 11.

Figs. 9–11. Calves 5396, 5437 and 5395, varying from 22 to 27 months old, showing severe East Coast fever reactions with death in two cases. The 'double' reactions shown by 5437 and 5395 are of particular interest.

schizonts appearing on the 23rd day. There was a period of apparent recovery followed by a severe fatal reaction from the 31st day until death on the 46th day, schizonts appearing in the blood for 14 consecutive days. Bovine 5395 showed a relatively severe initial reaction with the morning temperature above 104° F. for 9 days, but schizonts were only present on 1 day. This initial attack was followed by a mild secondary attack on the 40th–42nd day with schizonts present on the 40th, 45th and 46th days. This animal recovered.

The marked variation in the reactions seen in the remaining six younger animals are of interest in that they stimulate the types of reactions seen under field conditions. The most severe reaction followed by recovery was shown by B 5478 (Fig. 12), where the temperature was above 104° F. for 9 days and schizonts were present intermittently for 10 days. Mild reactions with schizonts showing for only 1–3 days, and temperatures above 104° F. for 5, 2 and 2 days were shown in calves 5575, 5469 and 5566 respectively (Fig. 13). Bovine 5464 showed the clinical signs and temperature reaction of a *Theileria parva* infection with numerous small piroplasms present in the erythrocytes but *no* schizonts were found (Fig. 14).

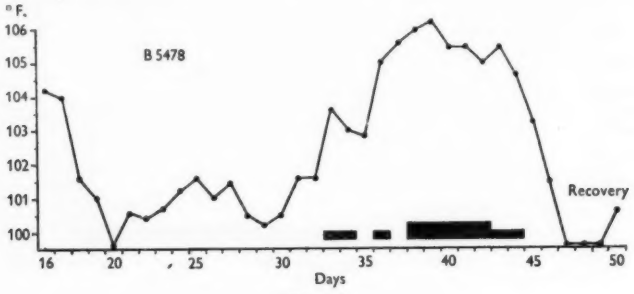


Fig. 12.

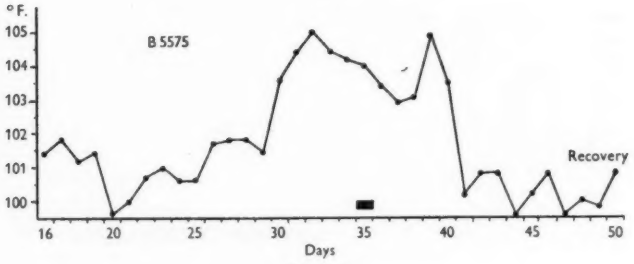


Fig. 13.

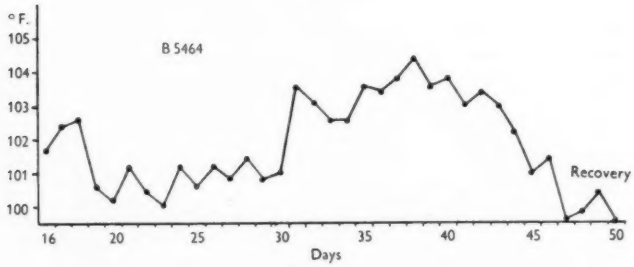


Fig. 14.

Figs. 12–14. Fig. 12. Calf 5478, a severe non-fatal East Coast fever reaction with intermittent appearance of schizonts. Fig. 13. Calf 5575, a type of non-fatal reaction with Koch's bodies rare as shown also by calves 5469 and 5566. Fig. 14. Calf 5464, an East Coast fever reaction without Koch's bodies appearing in gland smears.

The reaction shown by B 5573 is also of interest (Fig. 15). The course of the *T. parva* infection was very mild with a very low temperature reaction and schizonts present in gland smears for only 3 days. This infection *per se* appeared therefore insufficient to cause death. This animal was, however, suffering from a heavy untreated *Haemonchus contortus* infection and was in very poor condition when put on experiment, a condition which in this case appeared to aggravate the *Theileria parva* reaction.

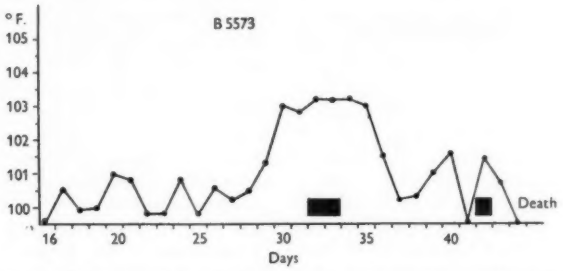


Fig. 15. Calf 5573, a mild East Coast fever reaction accompanied by severe *Haemonchus contortus* infection causing death.

Table 4. *East Coast fever reactions in four susceptible calves following* Theileria mutans *infection*

Bovine no. and sex	Age (months)	Day after exposure. Temp. above 103°F. with swollen glands	Max. temp. during reaction	Days on which K.B.B. found in gland smears	Days on which *Theileria* found in blood	Result
5506♀	22	25th day (19 July)	106°F. on 30th day	28th–37th day	Scanty *Theileria* present 40 days approx.	Recovered
5614♀	24	22nd day (16 July)	105·6°F. on 33rd day	25th–40th day	Scanty *Theileria* 60 days approx.	Recovered
5528♀	20	102·8°F. on 26th day (20 July)	Never above 102·8°F.	31st and 32nd days	Scanty 50 days approx.	Recovered
5509♀	22	13th day	105°F. on 16th day	Nil	Scanty *Theileria* present in blood 60 days approx.	Recovered

A further feature of interest, a feature also seen in calves 5465 and 5467 (Figs. 6, 7) in the previous experiment, was the apparent 'double' reaction shown by calves 5437, 5395 and 5573, and the intermittent appearance of schizonts in the case of no. 5478. As this type of reaction was not noted when single batches of infected ticks were fed on animals, it may be assumed that the second or third appearance of schizonts in each case is due to a reinfestation of infected ticks. This aspect will be dealt with more fully in a later paper dealing with immunity.

Bovines 5575, 5464 and 5469 were tested for immunity on 31 October 1947, 12 months approximately after the original reaction. Four infected nymphs (batch no. 51) were fed on each animal, and all three calves proved resistant. A susceptible calf, no. 5683 on which three nymphs from the same batch of ticks were fed, died of East Coast fever.

(3) *East Coast fever reactions in calves showing* Theileria mutans *infection*

Four heifer calves were grazed in a tick-infested paddock (paddock no. 3) from 1 April until 25 June 1947. The tick population infected with *T. parva* had previously been reduced in this paddock by grazing a number of immune animals, and the calves were further protected by a thorough overall spraying with a D.D.T.-Coumarone resin emulsion.

No *T. parva* infections occurred, but all four animals later in June showed some small piroplasms in the blood with anaemia and anisocytosis. As there was no rise in temperature and no schizonts were found in gland smears, a *T. mutans* infection was diagnosed.

On 25 June the calves were put to graze in the same tick-infested paddock used in the previous experiments (paddock no. 4). On the first day of temperature rise, each animal was removed, sprayed with D.D.T. and stabled. The tick counts during the period of grazing remained low, never more than three or four attaching at any one time. As the number of infected ticks had been reduced this total tick count was now no longer significant. The result of this experiment is summarized in Table 4.

Again there was a variety in the type of reaction and in the 'incubation' periods (Figs. 16–19). Calf 5509 reacted after a normal exposure of 13 days, and the delay in the onset of symptoms in the other three animals emphasized the scarcity of *T. parva* infected ticks now present in this paddock. Calves 5506 and 5614 developed relatively severe reactions, whereas in 5528 and 5509 the attack was relatively mild. These results were comparable to those in the two previous experiments, in that when few infected ticks are present there is a tendency towards more mild reactions with a greater percentage recovery. The presence of *T. mutans* did not appear to affect the course of the reactions. All four calves showed a solid immunity when tested with infected *Rhipicephalus appendiculatus* nymphs in October 1947.

(4) *East Coast fever reactions when infected ticks are reduced to a minimum*

Throughout 1947 the number of infected ticks in the paddock used in these experiments was being continually reduced. As previously noted, immune animals were grazed for short intervals, susceptible animals on experiment were removed early in the reaction, and at times the total ticks were reduced by use of tickicidal sprays or manual deticking. As a result, although a tick population of *R. appendiculatus* was maintained, the number of infected ticks became reduced. In the final experiment in 1947, three calves were exposed in this paddock on 1 December. Two, which had previously shown mild reactions, were now being tested for immunity. They showed no reaction during this exposure, but the third calf, no. 5672, 9 months old, showed a mild non-fatal reaction (Fig. 20). Throughout the period of exposure, this calf carried a relatively high tick infestation, rising from three adult *R. appendiculatus* ticks on the 2nd day to 16 adult ticks on the 19th day. This mild reaction was undoubtedly due to a scarcity of infected ticks. This is the type of reaction often seen in endemic areas when the total mortality is not more than 5% and where young susceptible animals are grazed with older immune animals or with herds of sheep and goats. When tested for immunity in March 1948 this animal was immune.

(5) *East Coast fever reactions following partial tick control by feeding benzene hexachloride to calves*

When benzene hexachloride powder D 929 was fed to calves in various doses, the active isomer circulated in the blood of the calves for a number of days, and considerable protection was afforded against ticks and other ectoparasites (Wilson, 1948*b*). In one experiment six calves susceptible to East Coast fever were grazed with a native herd for 28 days on unrestricted bush-pasture infested with various tick species including *R. appendiculatus* and *R. evertsi* and where East Coast fever was known to occur. A summary of the dosage of benzene hexachloride and the resulting tick control and *Theileria parva* reactions are given in Table 5.

It would appear that the severity of the *T. parva* reaction depended on the tick control afforded, and this in turn depended on the amount and the frequency of the benzene hexachloride dosage. When tick protection was good, no reaction occurred. When control was only partial, mild reaction occurred, and these calves proved immune 11 months later. The control animal died of typical *T. parva* infection. Similar results could be expected if calves were manually deticked.

(6) *East Coast fever reactions when definite numbers of infected ticks were fed on susceptible animals*

In the previous experiments it was shown that the chances of provoking mild non-fatal reactions increased as the number of infected ticks was reduced. There was still, however, doubt as to the exact number of infected ticks which would cause fatal and non-fatal reactions; therefore, in this experiment, known numbers of infected ticks were fed on susceptible calves.

(*a*) *Feeding* 4, 3, 2 *and* 1 *infected ticks on calves*

Laboratory-bred *Rhipicephalus appendiculatus* larvae (batch no. 34) were fed on calf 5619 during the height of an East Coast fever reaction. The nymphs, on moulting, were fed on nine calves in varying numbers and the results are summarized in Table 6.

Four infected nymphs were fed on two calves and produced a typical fatal East Coast fever reaction.

One infected nymph was fed on calf 5630 on 4 July 1947, and although the tick engorged no detectable reaction followed. On 13 August, 40 days later, three nymphs were fed and engorged successfully. The temperature first rose above 103° F. on

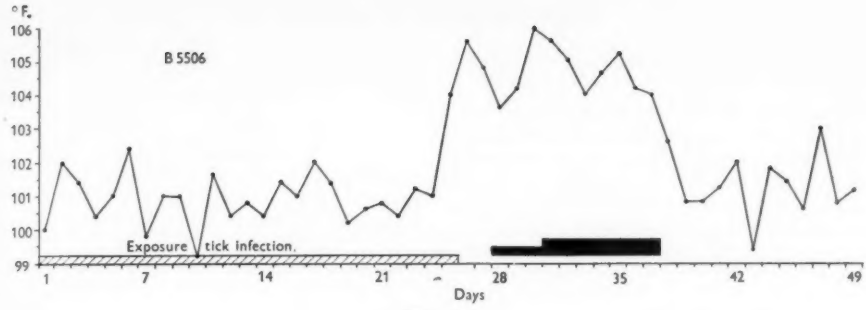


Fig. 16.

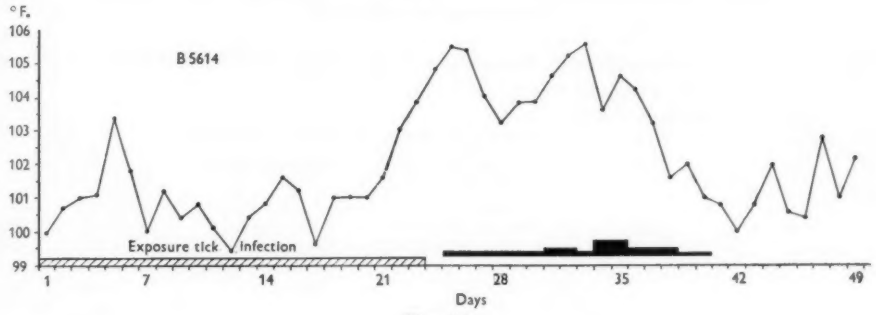


Fig. 17.

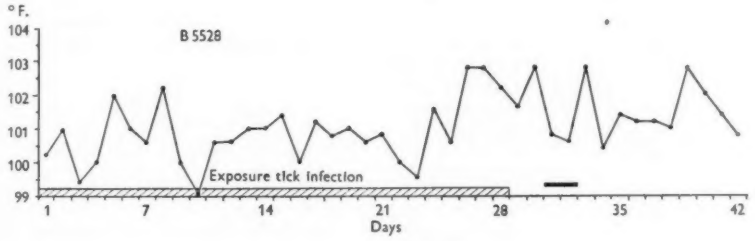


Fig. 18.

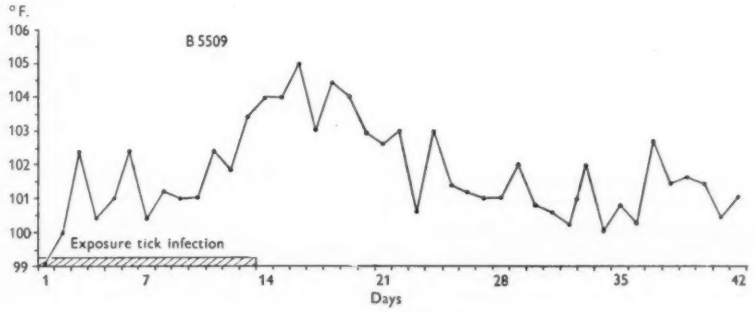


Fig. 19.

Figs. 16–19. East Coast fever reactions when infected tick population was low in infested paddock. Calves 5506, Fig. 16, and 5614, Fig. 17, showed severe reactions. Calf 5528, Fig. 18, showed a mild irregular temperature reaction with Koch's bodies present in gland smears for only two days. Calf 5509, Fig. 19, showed a reaction with no Koch's bodies present.

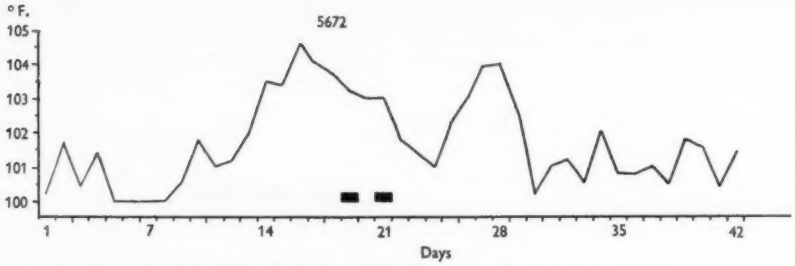


Fig. 20. Calf 5672, showing mild non-fatal reaction when infected ticks in pasture were reduced to a minimum.

Table 5. *East Coast fever reactions in five calves dosed with benzene hexachloride and one untreated calf*

Calf no.	Age (months)	Dose of benzene hexachloride D 929 (g./kg.)	Interval of dosage	Degree of control	Result
5568	25	0·25	On 1st, 7th, 14th and 21st days of exposure	Almost complete	No reaction
5571	12	0·25	On 1st and 14th days of exposure	Not complete. 17 ticks attached on 14th day and 5 on 28th day	First symptoms developed 14 days after last dosage. Temp. 104·8° F. on 1 day only. Very scanty K.B.B. on 4 consecutive days. Recovered
5574	12	0·125	On 1st, 4th, 7th, 10th, 13th, 16th and 19th and 22nd days of exposure	Almost complete	No reaction
5577	11	0·125	On 1st, 7th, 14th and 21st days of exposure	Good. Ticks failed to engorge	No reaction
5579	11	0·1	On 1st, 7th, 14th and 21st days of exposure	Not complete. Live ticks found on day of dosage	First symptoms developed 12 days after last dosage. Temp. over 104° F. for 3 days only. Very scanty K.B.B. for 3 days only. Recovered
5580	11	Untreated control	—	Nil. 32 ticks attached	Typical East Coast fever with death on 46th day

the 13th day and an acute East Coast fever reaction developed, but Koch's bodies were never numerous in gland smears and were absent for 9 days before death. Although the leucopaenia was severe, the white cell count did not fall below 1000 cells per cu.mm. as it did in most other fatal cases. Death occurred on the 31st day (Fig. 21).

Three nymphs were attached on calf 5661 on 13 August and remained attached and engorging for 11 days, an abnormally long period. The temperature first exceeded 103° F. on the 21st day, and death took place on the 42nd day in spite of two blood transfusions (Fig. 22).

Two infected ticks were fed on calf 5640 on 4 July 1947. Both ticks attached but took a considerable time to engorge, one being removed fully engorged on the 11th day and the second on the 14th day. There was no reaction.

Three infected nymphs were fed on calf 5649 on 4 July, but only two attached and engorged and dropped off on the 6th day. No East Coast fever reaction occurred.

Table 6. *The results of feeding* 1–4 *infected ticks on susceptible calves*

Calf no.	Age (months)	No. of nymphs engorged	Age of nymphs on attachment (days)	Result
5625	11	4	6	Death of acute East Coast fever
5629	10	4	6	Death of acute East Coast fever
5630	10	3	46	Death of acute East Coast fever
5661	7	3	46	Death of acute East Coast fever
5640	9	2	6	No reaction
5649	9	2	6	No reaction
5587	16	1	6	No reaction
5670	5	1	6	No reaction
5653	10	1	46	No reaction

(b) Feeding 3, 2 *and* 1 *infected ticks on susceptible calves*

A similar type of experiment was conducted on a smaller scale during October 1947. An unrelated generation of *R. appendiculatus* larvae were used (batch no. 51), and these were fed on calf 5661 used in the previous experiment so that the same strain of *Theileria parva* was again transmitted. The results are summarized in Table 7.

Table 7. *The results of feeding* 1–3 *infected ticks on susceptible calves*

Calf no. and sex	Age (months)	No. of infected nymphs engorged	Age of nymphs on attachment (days)	Result
5683 ♂	6	3	29	Death of acute East Coast fever
5667 ♂	8	2	15	No reaction
5673 ♂	7	1	25	No reaction
5680 ♂	7	1	25	No reaction

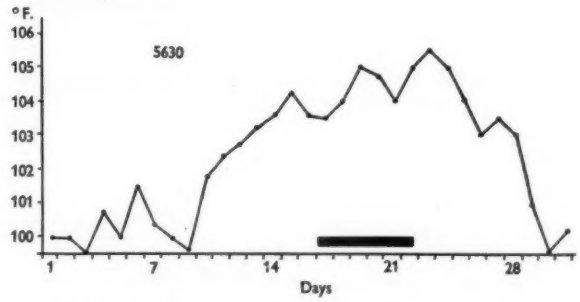


Fig. 21. Calf 5630, a fatal East Coast fever reaction produced by three infected ticks following the engorgement of one infected tick 40 days previously. Schizonts were present for only 6 days.

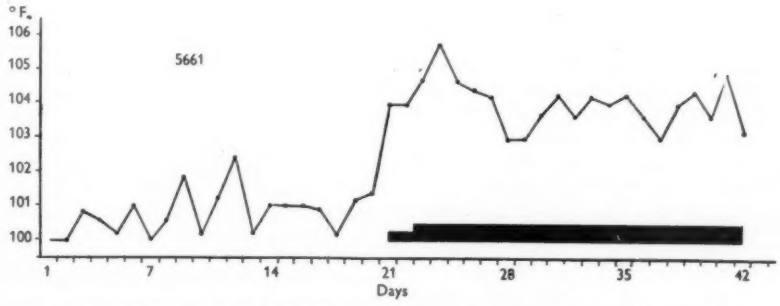


Fig. 22. Calf 5661, a fatal East Coast fever reaction caused by three infected ticks.

One nymph engorged successfully on each of the three calves 5587, 5670, 5653, but there was no detectable East Coast fever reaction.

One infected nymph 25 days after moulting was attached by means of an ear-bag to calf 5683 on 30 October but did not engorge. Three further

nymphs 29 days old were fed on 4 November. All three nymphs attached and two, fully engorged, were removed on the 6th day and one on the 7th day. The calf reacted on the 16th day and died 10 days later with Koch's bodies present in gland smears for 10 days and in blood smears for 4 days (Fig. 23).

Two infected nymphs 15 days old were fed on calf 5667 on 20 October and engorged successfully, one being removed fully engorged on the 26th and the other dropped off on 27–28 October. There was no reaction.

One infected nymph, 25 days old, was fed on each of two calves, 5673 and 5680, but the animals showed no reaction.

Again one and two infected nymphs failed to produce any detectable *Theileria parva* reaction. When three ticks were fed the reaction was comparatively mild (Fig. 24). Even this mild reaction, however, gave a serviceable immunity, since when six infected nymphs of the same Kabete batch of ticks were fed late in January 1948, a few days after the above reaction subsided, there was no second reaction.

This fact does not prove that the strain was more mild than the Entebbe strain. The result may be fortuitous or due to the age of the ticks when fed. It does, however, lend support to the findings of Lewis & Fotheringham (1941, p. 259), who report a mild infection in one bovine on which three ticks fed.

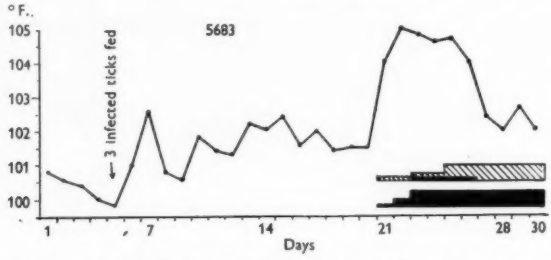


Fig. 23. Calf 5683, a fatal East Coast fever reaction following the engorgement of three infected ticks, Entebbe strain.

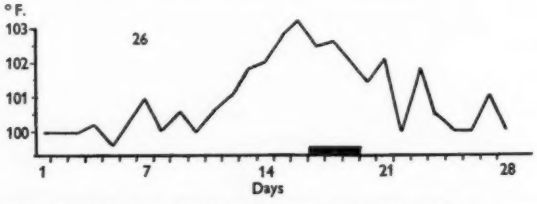


Fig. 24. Calf 26, mild East Coast fever reaction following the engorgement of three infected ticks, Kabete strain.

(c) *Feeding* 3, 2 *and* 1 *infected ticks* (*Kenya batch* 4858/101).

A batch of infected *Rhipicephalus appendiculatus* nymphs 4858/101, hatched on 9 May 1947, was received from Kabete Veterinary Laboratory on 19 December 1947. These ticks were fed on seven calves on 20 December and were therefore 225 days old. The results are summarized in Table 8.

Table 8. *Kenya ticks* (*batch* 4858/101)

Bovine no.	Age (months)	No. of ticks	East Coast fever reaction
26	13	3	Mild with recovery
5703	6	2	Nil
5678	10	2	Nil
35	8	2	Nil
5679	10	1	Nil
5697	7	1	Nil
5688	8	1	Nil

SUMMARY

Doubt exists as to why, in endemic areas, a varying proportion of calves die of East Coast fever while others survive. A series of experiments was therefore conducted to ascertain if the number of infected *Rhipicephalus appendiculatus* engorging simultaneously on susceptible calves influenced the course of East Coast fever reactions. The ages of the calves used in the various experiments varied, but other conditions were kept as uniform as possible.

When ten calves were grazed in a 1-acre paddock where the number of infected *R. appendiculatus* was high, eight died of acute East Coast fever.

In the second experiment, seventeen calves in all were grazed in the same paddock. These calves were given some protection from infected *R. appendiculatus* infestation, as two of the calves were immune to East Coast fever and the ears of ten of the susceptible

calves were sprayed with tickicidal solutions. Four died from acute East Coast fever and one of subacute East Coast fever complicated with a helminth infection.

In the third and fourth experiments, when four and three calves respectively were grazed in the same tick-infested paddock after the number of infected ticks had been further reduced, mild non-fatal reactions occurred in all the susceptible calves.

When benzene hexachloride was fed to susceptible calves exposed to tick infection in an endemic area the severity of the East Coast fever reactions depended on the tick control afforded.

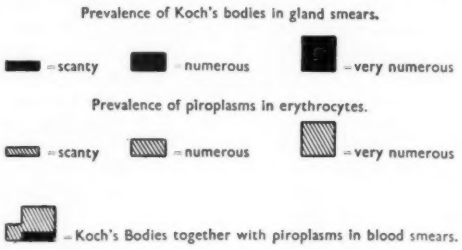
When known numbers of infected ticks were fed, four ticks and three ticks Entebbe strain fed simultaneously on susceptible calves, caused death, but two ticks and one tick caused no reaction. In a small experiment with ticks infected with Kenya (Kabete) strain of *Theileria parva*, three ticks caused only a mild reaction and two ticks and one tick again caused no reaction.

There was an indication in Exp. 2 that animals of 2 years of age and over reacted more severely than younger calves, but this was not supported by the results of Exp. 1. In the direct feeding tests three or four infected ticks Entebbe strain caused fatal reaction in young calves. If the initial tick infestation therefore is high, the age factor may not influence decisively the course of the reaction.

Attention is drawn to the intermittent appearance of Koch's bodies in gland smears during the course of certain East Coast fever reactions in cattle exposed in the tick-infested paddock producing so-called 'double' or 'triple' reaction. This is attributed to the successive maturing of parasites introduced by infected ticks on different occasions during the period of exposure.

My thanks are due to the Director of Veterinary Services for his permission to carry out this work and to publish this paper.

Key to temperature charts for Papers I and II



REFERENCES

BRUCE, D. (1910). 'Amakebe.' A disease of calves in Uganda. *Proc. Roy. Soc.* B, **82**, 35.

FOTHERINGHAM, W. & LEWIS, E. ANEURIN (1937). East Coast fever; its transmission by ticks in Kenya. *Parasitology*, **29**, 504.

HORNBY, H. E. (1934). *Annual Report of the Department of Veterinary Science and Animal Husbandry, Tanganyika Territory for* 1933. Dar-es-Salaam: Government Printer.

LEWIS, E. ANEURIN & FOTHERINGHAM, W. (1941). The transmission of *Theileria parva* by ticks. *Parasitology*, **33**, 251.

METTAM, R. W. M. (1933). *Annual Report of the Veterinary Department for the year* 1932, p. 31. Entebbe: Government Printer.

METTAM, R. W. M. & CARMICHAEL, J. (1936). Turning sickness, a protozoan encephalitis of cattle in Uganda. Its relationship with East Coast fever. *Parasitology*, **28**, 254.

WILSON, S. G. (1944). Theileriasis in cattle in Northern Province, Nyasaland. *Vet. Rec.* **56**, 255.

WILSON, S. G. (1945). Some factors affecting the incidence of East Coast fever in Northern Province, Nyasaland. *J. S. Afr. Vet. Med. Ass.* **16** (2), 47.

WILSON, S. G. (1946). Ticks and tick-borne diseases in Nyasaland. Ph.D. Thesis, Edinburgh University.

WILSON, S. G. (1948*a*). A method of assessing the tickicidal properties of D.D.T. and 'Gammexane' preparations in field trials. *Bull. Ent. Res.* **39**, 269.

WILSON, S. G. (1948*b*). The feeding of 'Gammexane' and D.D.T. to bovines. *Bull. Ent. Res.* **39**, 423.

(*MS. received for publication* 2. VII. 1948.—Ed.)

AN EXPERIMENTAL STUDY OF EAST COAST FEVER IN UGANDA

II. THE DURABILITY OF IMMUNITY IN EAST COAST FEVER

By S. G. WILSON, *Veterinary Research Laboratory, Entebbe, Uganda*

(With 5 Figures in the Text)

It is an established fact that an attack of East Coast fever in bovines followed by recovery can produce a strong immunity. This immunity differs from that caused by other protozoan parasites in that it is a sterile immunity, and du Toit (1931) showed that relapses did not occur in the absence of infected ticks. Doubt, however, has always existed as to whether immunity following one attack was durable throughout life or if it required to be reinforced by subsequent reinfection. In experimental work conducted at Entebbe a wide variety of reactions varying from acute to subacute have been produced (Wilson, 1949). In all the recovered animals the immunity following these attacks appeared durable in that the animals resisted further reinfection from 3 to 12 months later. The immunity following some of the mild transient infections is the subject of this paper.

EXPERIMENTAL RESULTS

(*a*) Two calves, a 12-months-old bull calf no. 5584 and a 16-months-old heifer calf no. 5553, were grazed on 31 March 1947, in a tick-infested paddock where the number of infected ticks was low. Both calves showed the early symptoms of East Coast fever on 1 May, the parotid and the prescapular glands being swollen and there was a temperature reaction in calf 5584. The calves were immediately removed from the paddock and were thoroughly sprayed with a D.D.T. emulsion to kill all ticks then attached but the reactions did not develop. Koch's bodies were found for only 1 day in the gland smears taken from no. 5584 and none was found in 5553 and small piroplasms were scanty in blood smears (Fig. 1*a*,*c*).

On 8 July, 38 days after the first reaction, four infected *Rhipicephalus appendiculatus* nymphs were

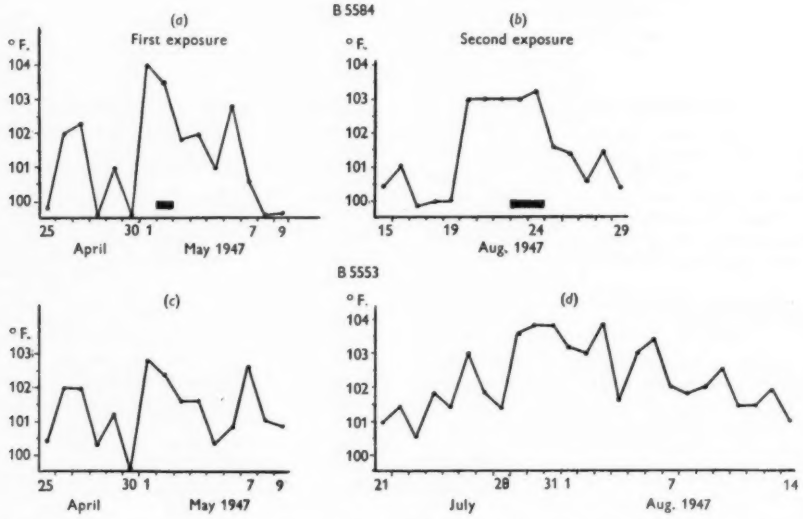


Fig. 1. Temperature chart of calves 5584 and 5553 showing mild initial East Coast fever reaction followed by second reaction 3 months later.

attached and engorged fully on calf 5553. Eighteen days after attachment of these ticks, the superficial glands became swollen, small piroplasms were present in the blood but the temperature did not exceed 104° F. and no Koch's bodies were found (Fig. 1*d*). Calf 5584 was grazed in the tick-infested paddock and showed a mild but definite East Coast fever reaction from 20 to 24 August (Fig. 1*b*).

On 11 October a further five infected ticks were attached and engorged on both calves, but throughout a 4-weeks observation period there was no glandular or temperature reaction and no leucopaenia. The calves were further tested for immunity on 24 December 1947. Six infected adult *R. appendiculatus* ticks received from Kabete Veterinary Laboratory, batch no. 4903/64, moulted on 29 September, were fed on each calf. Four of the ticks attached and engorged on calf 5553 and all six on calf 5584. There was again no reaction.

(*b*) A further instance of reinfection was noted in calf 5513, aged 23 months. This calf was grazed in a tick-infested paddock from 16 to 28 June 1947, the tick infestation being low, as the maximum number of ticks attached on any one day was 2♂♂ and 2♀♀ *R. appendiculatus* adults. Only a small proportion of these ticks were suspected of being infected with *Theileria parva*. The reaction following this exposure was very indefinite. The incubation period was prolonged. The maximum temperature was 102·7° F. on 24 July, the lymph glands were only slightly swollen and scanty Koch's bodies were found in gland smears for only two days (Fig. 2*a*). This animal was re-exposed in the tick-paddock from 18 to 20 August, the tick count on the 20th being 2♀♀ and 2 nymphal *Rhipicephalus appendiculatus*. The East Coast fever reaction following this exposure was more definite, the first signs appearing 13 days after exposure (Fig. 2*b*), the temperature remaining above 104° F. for 8 days with a maximum of 106·2° F. on 31 August. Although Koch's bodies were seen in gland smears for 9 days, the leucopaenia was not marked, the lowest count being 6600 cells per cu.mm. on 10 September. From work at present in progress, it has been observed that in an initial attack in fully susceptible calves the leucocytic count falls to 3000 cells per cu.mm. or lower when Koch's bodies are present from 6 to 8 consecutive days. It did appear, therefore, that as a result of the previous attack, the body mechanism of calf 5513 was able to deal with this relatively severe attack without the development of any severe leucopaenia.

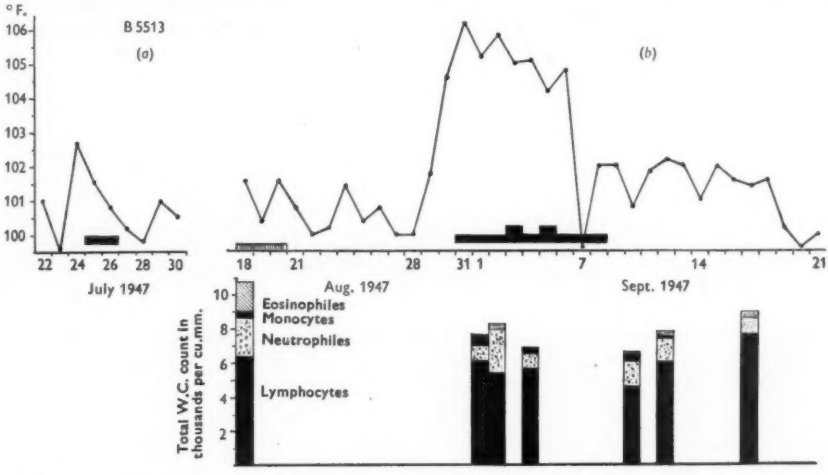


Fig. 2. Temperature chart of calf 5513 showing mild initial East Coast fever reaction followed by a more severe second reaction one month later.

(*c*) Further light on the duration of the immunity after mild infections is given by calves 5527 and 5529. These two animals, when grazing with the farm herd, became accidentally infected in May 1946, both animals being then 7 months old. The infected-tick infestation was low, as the calves were being dipped at that time and the majority of the other cattle in the herd were tick-free. The temperature reaction of B 5529 (Fig. 3*b*) was more severe than that of B 5527 (Fig. 3*a*), the temperature exceeding 104° F. for 8 days in the former animal as compared with only 1 day in the latter, but Koch's bodies were only found on one day in each case, i.e. 31 May. On 11 October 1947, 16 months approximately after the first attack, three infected *R. ap-*

pendiculatus nymphs 6 days after moulting ex batch no. 51, were fed on each animal. B 5529 gave no definite reaction, there being only slight glandular swellings and a temperature of 103° F. for 1 day. B 5527 reacted severely with a temperature of 104° F. or over for 12 days, with Koch's bodies present in gland smears on 16 consecutive days. There was November 1947 when 12 months old. The temperature reaction was relatively mild (Fig. 4*a*), and Koch's bodies were found on only 4 days. When exposed to reinfection in January 1948 a second mild attack occurred (Fig. 4*b*), less severe than the first, with Koch's bodies only showing for 2 days. Unfortunately, this animal died from a heavy

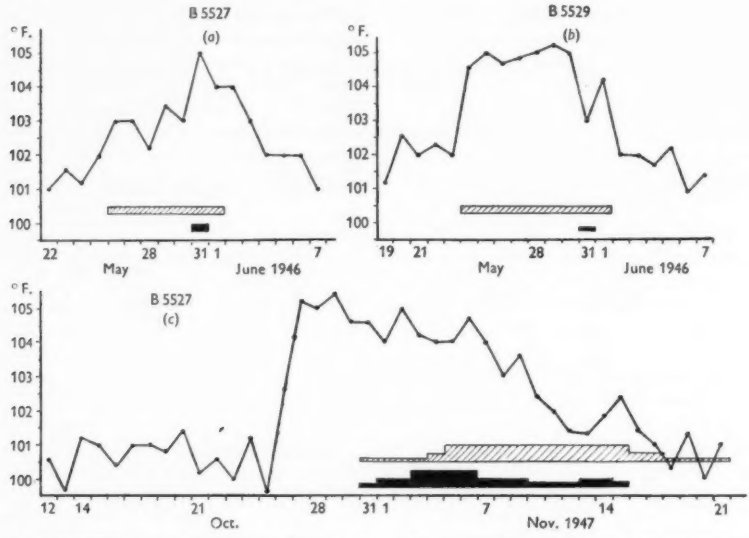


Fig. 3. Calf 5527. *a*, *c*, showing a mild East Coast fever reaction in May and June 1946 and a severe reaction 16 months later. The reaction of calf 5529 (*b*) was more severe in 1946, and this calf was still immune 16 months later.

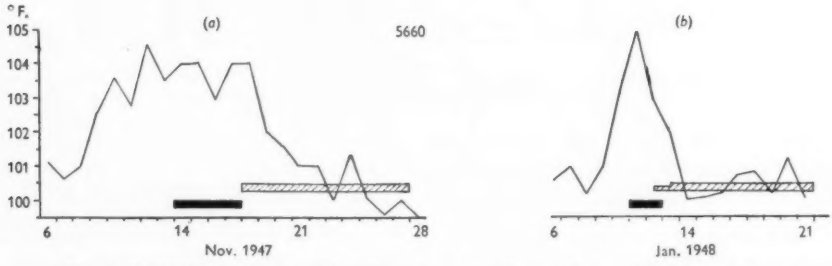


Fig. 4. Calf 5660 showing two East Coast fever reactions with a period of 3 months.

marked anaemia and leucopaenia, the erythrocytic count being 2·80 millions per cu.mm. on 17 November and the leucocyte count 1950 cells per cu.mm. on 5 November. This was the only animal in the present experiments to recover after being infected by three infected ticks. One might again assume that in this case some slight residual immunity existed from the previous attack in 1946.

(*d*) Heifer calf 5660 was accidentally infected in helminth infection before it could be retested for immunity.

DISCUSSION

Considerable confusion exists regarding the duration of the immunity produced in bovines by an attack of East Coast fever. Restricting attention to the East African infections, Walker (1928) stated that 'the immunity conferred by recovery from a natural

attack of East Coast fever is not necessarily absolute and complete against natural reinfection'. Daubney (1933) showed that eight adult bovines, known to be immune, did not lose their immunity after being kept free from reinfection for prolonged periods. Purvis (1935) believed that immunity could only be maintained in calves by repeated reinfection, and that protection from reinfection for as short a period as 7 weeks rendered them susceptible to fresh infection. Piercy (1947) reported a breakdown in immunity in a calf 2 years after an original infection, although the calf had remained throughout that period in an enzootic East Coast fever area.

The present work at Entebbe suggests that the severity of the attack and the durability of the immunity depends on the rate of infestation by infected ticks. When the infected tick population is sufficiently high to ensure that three or more infected ticks are engorging simultaneously, the reactions in susceptible bovines will be severe and the mortality rate high. If the rate of infection is low so that infected ticks attach singly or in pairs over varying intervals, the severity of the reactions tend to become less acute.

Immunity following acute and subacute attacks appears durable. In previous experiments (Wilson, 1949), all the animals tested, including such mild subacute reactors as calves 5528, 5509 and 5672 (figs. 18–20), were immune when tested 3–4 months afterwards. In the present series, the subacute reaction shown by calf 5529 produced sufficient immunity to protect against reinfection 16 months later. Future work will show whether this immunity is now absolute. The immunity following the very mild reactions shown by the remaining calves in the present series was only transient and reactions occurred on reinfection. The probable course of events in infected areas is shown diagrammatically in Fig. 5.

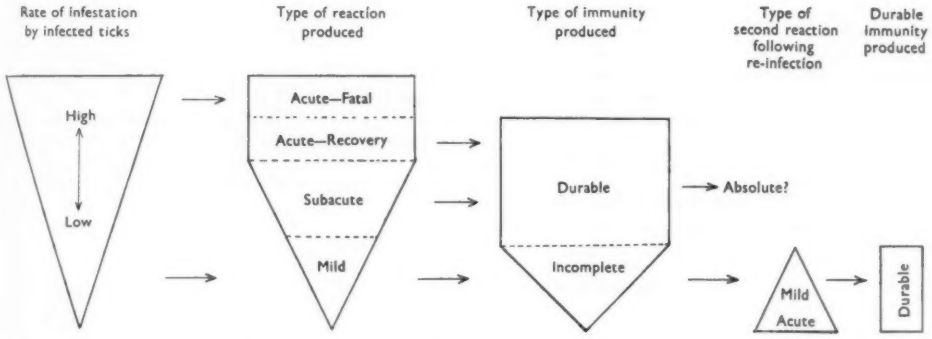


Fig. 5. Probable course of East Coast fever infections in enzootic and endemic areas. The severity of the first attack depends on the number of ticks engorging simultaneously. The durability of the immunity depends in turn on the severity of the attack.

The severity of the second reaction will no doubt depend, not only on the number of infected ticks which engorge, but also the time which has elapsed between the first and second attacks. When the interval was prolonged to 16 months in 5527 the second reaction was severe (Fig. 3c). With the other calves the interval was from 1 to 3 months, and the second reactions were never severe. The influence of shortening the interval between the reactions to a few days may be judged from the 'double' reactions previously noted. Thus calf 5437 (Wilson, 1949, fig. 10) had a mild attack from the 18th to 20th days, with schizonts in gland smears on the 23rd day. This first attack was followed by a more severe and prolonged fatal attack from the 30th to 46th days. The immunity following the first attack was either not strong or had not sufficient time to develop and was unable to prevent the second attack developed approximately 10 days later. The reverse was the case in calf 5395 (Wilson, 1949, fig. 11), where, following a severe attack from the 17th to 29th days, the mild attacks from the 40th to 45th days caused little upset.

Attempts to produce a third attack in our experimental animals has failed and, for the moment, it is assumed that a second attack gives a durable immunity.

At least three factors can be used to assess the severity of the East Coast fever reaction and the durability of the immunity likely to be produced, i.e. temperature reaction, leucopaenia and persistence of Koch's bodies. The persistence of Koch's bodies is not reliable, since immunity can be produced in their absence, e.g. calf 5553 (Fig. 1) and calves 5464 and 5509 (Wilson, 1949, figs. 14 and 19) and in calves

5527 and 5529 (Fig. 3) the schizonts were present for 1 day in each case, but the durability of the immunity was very different in each case. We have not as yet sufficient data on the leucopaenia produced by mild infections to use this feature as an immunity indicator at present. Comparing the original reactions of calves 5527 and 5529, the number of days the temperature is above 104° F. is at present the best indication of the severity of the infection and the type of immunity likely to be produced.

SUMMARY

Confusion exists as to whether the immunity produced by one attack of East Coast fever is absolute or whether it requires to be maintained by reinfection.

During the present experiments mild transient infections have been produced in six calves, and in five cases the immunity has been incomplete and second attacks have followed reinfestation by infected ticks.

The time interval between the attacks has varied from 1 to 16 months, and it is suggested that when the interval is prolonged the second attack will be severe and may be fatal.

It is concluded that the durability of the immunity depends on the severity of the original attack, being durable when the attack is severe, and only transient and probably never solid when the attack is mild.

The temperature reaction is at the moment the best indicator as to the severity of the attack and the type of immunity evolved.

My thanks are due to the Director of Veterinary Services for his permission to carry out this work and to publish this paper.

REFERENCES

DAUBNEY, R. (1933). *Annual Report of the Department of Agriculture, Kenya*, 1932, p. 289.

PIERCY, S. E. (1947). Immunity to East Coast fever. *Vet. Rec.* **46**, 636.

PURVIS, G. B. (1935). East Coast fever—A warning. *Vet Rec.* **15**, 280.

DU TOIT, P. J. (1931). Immunity in East Coast fever. *17th Report Dir. of Vet. Services and Research, S. Africa.*

WALKER, J. (1928). *Annual Report of the Department of Agriculture, Kenya*, 1927, p. 159.

WILSON, S. G. (1949). An experimental study of East Coast fever in Uganda. I. A study of the type of East Coast fever reactions produced when the number of infected ticks is controlled. *Parasitology*, **40**, 195.

(*MS. received for publication* 2. VII. 1948.—Ed.)

ON THE BEHAVIOUR AND SENSORY PHYSIOLOGY OF THE HOUSE-FLY LARVA, *MUSCA DOMESTICA* L.

I. FEEDING STAGE

By M. HAFEZ, M.Sc., Ph.D., F.R.E.S., *Department of Entomology, Faculty of Science, Fouad I University, Cairo*

(*From the Zoological Laboratory, University of Cambridge*)

(With 28 Figures in the Text)

CONTENTS

	PAGE
I. Introduction	215
II. General methods and material	216
III. Humidity reactions	217
(1) Methods	217
(2) Results	217
(*a*) Preliminary experiments with extreme alternatives	217
(*b*) Uniform humidities	217
(*c*) Alternative humidities	219
(*d*) Preconditioning	219
(*e*) The nature of the humidity stimulus and the effect of temperature on the humidity reaction	219
(3) Humidity receptors	220
(4) Mechanism of orientation	221
IV. Temperature reactions	223
(1) Methods	223
(2) Results	223
(*a*) Uniform temperatures	223
(*b*) Alternative temperatures	224
(*c*) Preconditioning	226
(3) Temperature receptors	226
(4) Mechanism of orientation	226
V. Reactions to smell	226
(1) Methods	226
(2) Results	227
(3) Mechanism of orientation	228
VI. Reactions to combinations of factors	228
(1) Two-factor experiments	229
(*a*) Temperature and Humidity	229
(*b*) Temperature and Light	229
(*c*) Temperature and Smell	229
(*d*) Humidity and Light	229
(*e*) Humidity and Smell	230
(*f*) Light and Smell	231
(2) Three-factor Experiments	233
(3) Four-factor experiments	233
VII. The reactions of the larva and the normal environment	233
VIII. Summary	234
References	235

I. INTRODUCTION

The reactions of the house-fly larva to four environmental factors, namely temperature, air humidity, smell and light have been studied in the Laboratory. It was hoped that the results would shed some light on the general behaviour of the larva in its normal habitat. A comprehension of this behaviour will assist in understanding the ecology of the species, and hence facilitate its control. In order to analyse the behaviour of the larva, its reactions (other than to light) were first studied in relation to one factor at a time. An attempt was then made to test experimentally its reactions to various combinations of factors.

From the literature available to the writer, it seems that there has been no work published on the reactions of fly larvae to air humidity. Recently, however, such reactions have been demonstrated in a few insects. For instance, in *Locusta migratoria* (Kennedy, 1937), *Culex fatigans* (Thomson, 1938), *Blatta orientalis* (Gunn & Cosway, 1938), *Tenebrio molitor* (Pielou & Gunn, 1940) and in *Pediculus humanus corporis* (Wigglesworth, 1941) it has been found that such responses lead to the avoidance of moist air. On the other hand, in wireworms the reverse takes place; the insects avoid the dry air and aggregate in moist air (Lees, 1943).

The reactions of the house-fly larva to temperature have also received little attention from previous workers. Thomsen & Thomsen (1937), however, investigated the thermo-preferendum of the larva in a temperature-gradient apparatus. In this apparatus, the larva was allowed to move in horse-dung so that conditions were as nearly as possible those prevailing in its normal environment. Ac-

cording to these authors, feeding larvae showed a definite temperature preference for the range 30–37° C. which corresponded with that found in their natural habitat in a fermenting manure heap. They also concluded from their experiments that the vertical distribution and movements of the larvae in natural conditions depend chiefly on their temperature preference, and to a lesser degree on chemotaxis, negative phototaxis, hygrotaxis and thigmotaxis. The relation between temperature and the rate of movement was investigated by Miller (1929) in the larvae of *Lucilia*. The rate of movement of the larva was found to be directly proportional to temperature between 2 and 40° C. and inversely so above 40° C. Also the rate of contraction of the larva increased directly with temperature from 0 to 45° C.

The larva is sensitive to smell. This was observed and experimentally demonstrated in both house- and blow-fly larvae by a number of workers, notably Herms (1911), Welsh (1937) and Bolwig (1946). Bolwig paid more attention to the subject, and carried out numerous experiments in an attempt to locate the larval olfactory organ. This he showed to be the two dorsal papillae (so called antennae) of the cephalic lobes as suggested by Welsh (1937).

On the response of fly larvae (e.g. those of *Calliphora*, *Lucilia* and *Musca*) to light, much valuable information is included in the works of Pouchet (1872), Lowne (1890–2), Loeb (1890, 1905), Holmes (1905), Mast (1911), Herms (1911), Patten (1914), Hewitt (1914), Crozier (1925–8), Mitchell & Crozier (1927–8), Ellsworth (1933), Welsh (1937), Fraenkel & Gunn (1940) and Bolwig (1946). While there is a general agreement among these authors that the larva is photo-negative, and that it can orient itself in relation to the source of light in such a way that its anterior end receives minimum and equal illumination on both sides, there has been a wide diversity of opinion as to the identity of the light sensitive organs. These have been proved by Bolwig (1946), however, to be two small groups of sense cells situated on each side just above the anterior ends (condyle spines of Bolwig) of the pharyngeal sclerites of the larval skeleton. As regards the mechanism of orientation of the larva to light it is a typical example of klinotaxis (Fraenkel & Gunn, 1940; Bolwig, 1946).

II. GENERAL METHODS AND MATERIAL

The apparatus used for studying the reactions of the larva was substantially the same as that described by Wigglesworth (1941). It consisted of two copper tanks, each 20 × 20 × 9 cm., separated by an asbestos sheet 2 mm. thick, and held together by means of an outer metal strip. Warm or cold water could be circulated through the tanks, or they could be heated from below with micro-burners.

In most of the experiments the larva was observed in a circular arena 10·5 cm. in diameter, and arranged in such a way that its two halves contained two different intensities of a single factor at a time. The floor of the arena was of bolting silk stretched on a perforated zinc platform. Except in the experiments on humidity reactions, the arena had metal walls 2·5 cm. in height. The arena rested on one tank, except in the experiments on temperature reactions which was heated to give a temperature of about 25° C. on the floor of the arena. The temperature was measured by a sensitive resistance thermometer ('skin temperature recorder'). In all but the light experiments the arena was near the window and shielded by a piece of brown paper from direct daylight. A single fresh larva was used for each experiment. Groups of larvae usually clump together and make the study of their responses very difficult. Ten to fifteen experiments were carried out to test the reaction of the larva to a given pair of alternative intensities of any one factor. Roughly half way through these experiments, the two intensities were interchanged between the sides of the arena in order to eliminate any possible bias of the insect toward one particular side. The duration of each experiment varied from 10 to 30 min. Over this range the duration of the experiment did not seem to have any appreciable effect on the intensity of the reaction. The tracks made by the larva during its movement on the floor of the arena were copied on a sheet of paper alongside the apparatus and, with the aid of a stop-watch or an audible time signal, half minutes were marked on the tracks.* Analysis of these tracks revealed any differences in the length of time spent by the larva on the two sides, and showed whether these were due to an orthokinetic reaction (i.e. to a difference in the rate of forward movement of the larva), or to a klinokinetic reaction (i.e. to a difference in its rate of change of direction), or due to a directed reaction or taxis.

This method was used for studying the humidity reactions of *Porcellio* (Gunn, 1937), *Locusta* (Kennedy, 1937), *Blatta* (Gunn & Cosway, 1938), *Culex* (Thomson, 1938), *Tenebrio* (Pielou & Gunn, 1940, wireworms (Lees, 1943), and *Ptinus* (Bentley, 1944) and for studying the sensory physiology of *Pediculus* (Wigglesworth, 1941), *Ixodes* (Lees, 1948), and the temperature reactions of *Culex* (Thomson, 1938).

The apparatus described above has several advantages. For instance, it is possible in this apparatus to : (*a*) provide a very steep gradient along the boundary between the two halves of the arena;

* When the larva moved against the wall of the arena, its tracks were recorded for the sake of clearness as concentric lines.

(*b*) study the reactions of the larva to small differences of temperature or relative humidity; (*c*) easily arrange the two halves of the arena in such a way that one differs from the other in respect of two, three or four factors at a time; and (*d*) make a detailed study of the mechanisms of orientation of the larva.

Thirty to fifty fresh third-stage larvae were used every day for the experiments. They were all of nearly the same size and age (about 3 days old). They were reared on milk (Hafez, 1948) at a temperature of about 27° C. and a relative humidity of about 100 %. Care was taken that all experimental larvae were in the same physiological condition. Further details on the methods will be given in the separate sections below.

III. HUMIDITY REACTIONS

(1) *Methods*

The method used for studying the humidity reactions is nearly the same as that of Wigglesworth (1941). On the roof of the warm tank a glass rod 1·5 mm. in diameter was fastened by a strip of cellulose adhesive tape. On either side of it were placed four semi-circular thicknesses of blotting-paper soaked in 5 c.c. of the salt solution giving the required humidity. A zinc false floor, 11 cm. in diameter, covered with bolting silk, was supported about 1 mm. above the blotting-paper by six very small pieces of glass. The arena was enclosed by the lid of a Petri dish 9·5 cm. in diameter and 5 mm. deep. A glass rod, 2 mm. in diameter, embedded in wax, was fastened across the roof of the arena to minimize the area of contact between the air on each side. A larger Petri dish, 12 cm. in diameter, covered the entire apparatus so as to isolate the arena from the outside atmosphere. At high temperatures, the outer cover was slightly warmed before each experiment to prevent the condensation of water vapour on the inner cover.

The following salt solutions were used for controlling humidity at 25° C. (Adams & Merz, 1929; Buxton, 1931; Buxton & Mellanby, 1934):

K_2SO_4	98 %	$MgCl_2$	33 %
KCl	83 %	Neutral Na-tartrate	92 %
$Ca(NO_3)_2$	50 %	NH_4NO_3	62 %
KH_2PO_4	95 %	K-acetate	18 %
NaCl	75 %		

The humidities given by these salts were checked before each set of experiments. In many cases the same experiments were repeated by using concentrated sulphuric acid-water mixtures (Wilson, 1921).

It was assumed that the humidity above the floor was that given by the salt below, and that the steepest part of the humidity gradient would be in the zone along the median line.

Once set up, the apparatus was left about half an hour for the humidity equilibrium to become established, and then the larva was introduced by slightly lifting the cover. The disturbed equilibrium would be re-established soon after, according to Sweetman (1933).

The intensity of reaction was expressed when required, as the excess percentage $100\left(\frac{W-D}{W+D}\right)$ (Gunn & Cosway 1938). W and D are the times spent by the larva on the wetter and drier sides respectively. Time spent in the middle zone was ignored.

When sulphuric acid-water mixtures were used the apparatus consisted of two Petri dishes of equal diameters and depths (9 and 1 cm. respectively), one inverted on the other. The lower dish was divided in two equal compartments by a glass rod embedded in wax. The desired concentrations of sulphuric acid were placed in these compartments. The upper dish forming the arena was similarly divided and rested on a zinc-silk platform. The rim of the upper dish lay exactly above that of the lower dish. The whole apparatus was covered by a larger Petri dish. Experiments with this apparatus were carried out in a constant temperature room at 25° C.

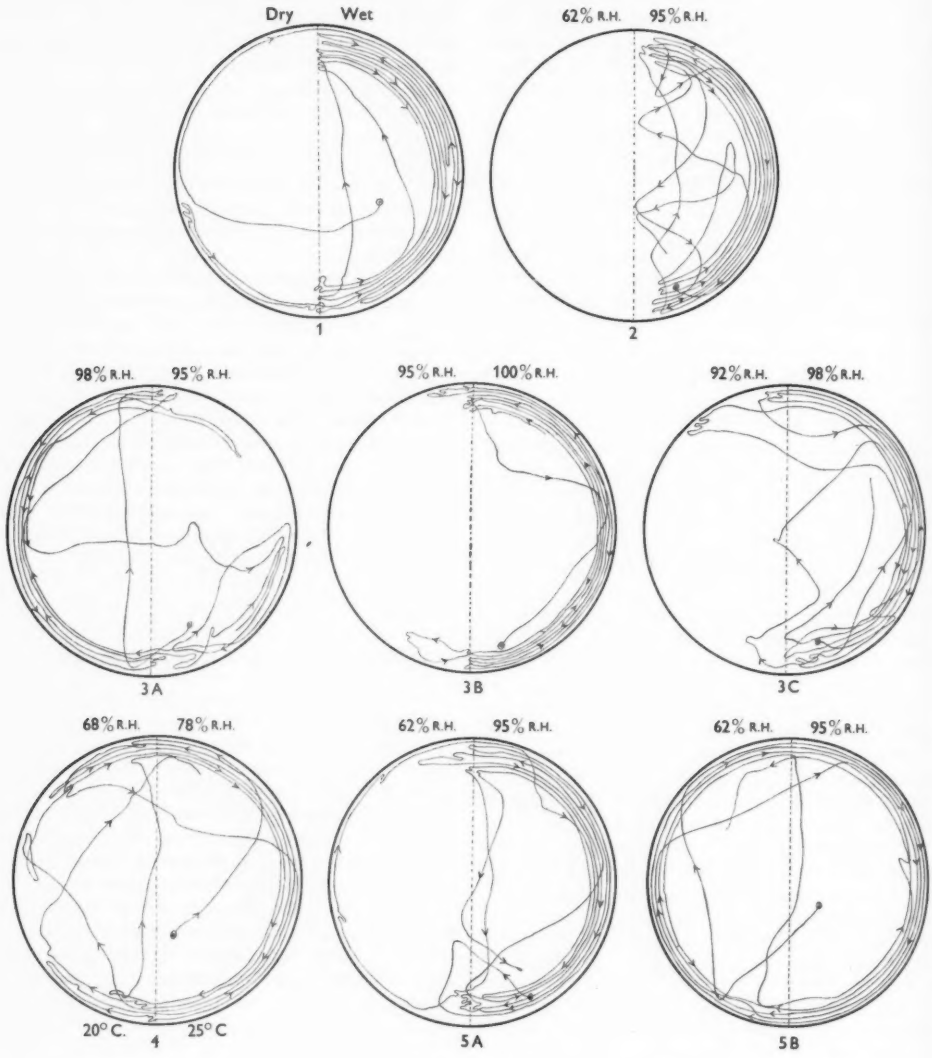
(2) *Results*

(*a*) *Preliminary experiments with extreme alternatives*

Before studying reactions to air humidity in the apparatus described above, the insects were offered the choice of wet and dry surfaces to move on. This was done by dividing a circular sheet (15 cm. in diameter) of blotting-paper into two equal halves. One half was saturated with water, the other left dry, and the arena left open above. Fig. 1 represents a typical larval track in this arena. This larva persistently avoided the dry side and spent nearly all its time on the wet side. The same result was obtained when the larva was offered the choice of dry air and saturated air brought about by concentrated sulphuric acid and water respectively.

(*b*) *Uniform humidities*

A series of control experiments was carried out in which the behaviour of the larva was recorded at uniform humidities. The following relative humidities were used, each in one experiment: 33, 62, 75, 95 and 98 %. At the lowest humidity (33 %) the larva changed its course frequently, while at higher humidities (75 or 98 %) the larva walked straight round the wall of the arena. There was also a difference in the rate of movement of the larva. One larva, for instance, moved at an average rate of 14 cm./min. at 33 % and 9 cm./min. at 98 % R.H.



Figs. 1–5.

Fig. 1. Reaction of larva to a dry surface.

Fig. 2. Reaction of larva to air humidity.

Fig. 3A, B, C. Reactions of larvae to humidity differences within the range from 90 to 100 % R.H.

Fig. 4. Track followed by larva showing a fairly strong reaction to lower humidity, the saturation deficiency being the same on each side of the arena.

Fig. 5A, Track followed by decapitated larva showing marked avoidance of lower humidity; **B,** track of larva whose body (except the head) was covered with vaseline, showing indifference to humidity.

(c) *Alternative humidities*

Fig. 2 shows the typical behaviour of a larva given a choice of relative humidities of 62 and 95 %. The larva spent all its time on the moist side, showing a marked avoidance of the lower humidity. The intensity of the reaction expressed as excess percentage on the moist side was thus 100 %. Larvae on approaching the mid-line often turned back when 1 cm. or more distant from it. Sometimes, however, this failed to bring them back into the moist side, and they then continued to move agitatedly on the drier side, pursuing an irregular course.

The following pairs of alternative humidities were also used:

100 or 98 %,	100 or 95 %,	100 or 92 %,	98 or 95 %,
98 or 92 %,	95 or 92 %,	95 or 83 %,	95 or 75 %,
95 or 62 %,	95 or 50 %,	95 or 33 %,	92 or 83 %,
92 or 75 %,	92 or 62 %,	92 or 50 %,	92 or 33 %,
83 or 75 %,	83 or 62 %,	83 or 50 %,	83 or 33 %,
75 or 62 %,	75 or 50 %,	75 or 33 %,	62 or 50 %,
62 or 33 %,	62 or 18 %,	50 or 33 %,	50 or 18 %,
50 or 10 %,	33 or 18 %,	35 or 10 %,	20 or 10 %.

It was found that below 50 % R.H. larvae seemed quite unaffected by differences of 25 % or even 30 % R.H. For example, larvae showed no preference when offered alternatives of 10 or 25, 10 or 35, 25 or 50, 18 or 50 % R.H. But a weak reaction was obtained when they were offered 10 or 50 % R.H.

Above 50 % R.H., the larva showed a consistent avoidance of the drier side. The intensity of this avoidance depended on the humidity range of the alternatives offered: the higher the range, the greater the intensity. For instance, the reaction intensities to about the same humidity difference at 75 or 83, 83 or 92 and 92 or 100 % R.H. were 62, 78 and 100 % respectively. Within the upper range, from 90 to 100 % R.H. smaller humidity differences such as 2 % (at 98 or 100 % R.H.), 3 % (at 95 or 98 % R.H.), 5 % (at 95 or 100 % R.H.) or 6 % (at 92 or 98 % R.H.) were sufficient to produce a clear avoidance of the drier side (Fig. 3A, B, C). It seems, therefore, that in the presence of 100 % R.H. all lower humidities are avoided. The sensitivity of the larvae to humidity is greatest when the alternative offered is close to saturation.

If some allowance is made for the different apparatus used, it would appear that the humidity behaviour of the larva resembles in some respects that of wireworms. Thus Lees (1943) has shown that wireworms also avoid dry air and that an intense reaction appears only over the upper humidity range, the insects being indifferent to humidities below 70 % R.H. The disappearance of the response at lower humidity may be attributed to a failure in the operation of orientation mechanisms under adverse physiological conditions rather than a real indifference to humidity (Lees, 1943). Also in *Tenebrio* (Pielou & Gunn, 1940), in *Culex* (Thomson, 1938) and in *Pediculus* (Wigglesworth, 1941), which all avoid moist air, it has been observed that the sensitivity of the insect to humidity is greater within the higher ranges of humidity and especially near saturation.

(d) *Preconditioning*

The possible influence of the air humidity to which the larva had been previously exposed was tested in the following way. The larva was offered a choice of a higher relative humidity (95 % R.H.) and a lower relative humidity (33, 50 or 62 % R.H.). The higher humidity was kept constant at 95 % at one side of the alternative chamber. The lower humidity offered on the other side was the same to which the larva had been previously exposed for 5 hr. In almost all cases it seemed that preconditioning had no influence on the behaviour of the larva; preconditioned larvae preferred the moist side and avoided the dry. Some larvae, however, when offered 62 or 95 % R.H., avoided the higher humidity at the outset, but soon this reaction was reversed.

(e) *The nature of the humidity stimulus and the effect of temperature on the humidity reaction*

The effect of temperature on the humidity reaction has been studied in *Culex* (Thomson, 1938), in *Tenebrio* (Pielou & Gunn, 1940) and in wireworm (Lees, 1943). This was done by testing the behaviour of the insect at different temperatures when offered the same pair of alternative humidities. In *Culex* and *Tenebrio* it was found that the intensity of the reaction was more closely correlated with relative humidity than with saturation deficiency. On the other hand, saturation deficiency (that is the evaporating power of the air) seems to be the important factor in determining the reaction intensity of wireworms.

In order to find whether the humidity reaction of the house-fly larva is related to the saturation deficiency of the air or to its relative humidity the following experiment was carried out.

Larvae were offered a choice of 68 or 78 % R.H. in the alternative chamber, the temperature being 20° C on the drier and 25° C. on the moister sides.* As will be seen later, larvae are unaffected by a 5° C. temperature difference between 20 and 25° C. The calculated saturation deficiency on either side was the same (0·2 of an inch or 5 mm. Hg). Thus the only variable factor in the alternative chamber was the relative humidity. Fig. 4 shows a typical result. The

* 78 % R.H. at 25° C. and 68 % R.H. at 20° C. were produced by saturated solutions of NH_4Cl and NH_4NO_3 respectively.

larva spent most of its time on the moister side and avoided the drier side. These experiments suggest that the house-fly larvae respond to the relative humidity of the air rather than to its saturation deficit.

Another series of experiments in which the larvae were exposed to the same relative humidity choice at 20, 25 and 32° C. was carried out. The pair of alternative humidities used being 62 or 95% R.H. The intensity of reaction was nearly the same (about 82·5%) at 20 and 32° C., although the calculated difference in saturation deficiency at 32° C. was nearly twice that at 20° C. These results lend support to those obtained from the first series of experiments, and show that it is differences of relative humidity and not of saturation deficiency which seem to control the humidity behaviour of the larva.

(3) *Humidity receptors*

The striking avoidance of the dry side shown by the larva at 62 or 95% R.H. was made use of in an attempt to locate the humidity receptors. The cephalic lobes of the larva were tested first, as they are the seat of a number of sense organs (Bolwig, 1946). Larvae were therefore anaesthetized by carbon dioxide, and their lobes were covered by a thin coat of cellulose paint. But larvae got rid of this coat soon after recovery because of the secretion of large quantities of saliva and the continuous pressing of the head against the floor of the arena. Other covering substances such as shellac or a mixture of celloidin, acetone and resin were tried but all without success. Neither cauterization nor the use of 1% solution of cocaine hydrochloride as a local anaesthetic (Hartwell, 1924) gave satisfactory results. The elimination of the sense organs on the head was then tried by tying the cephalic lobes with a very fine silk thread and cutting them with fine scissor-forceps (Bolwig, 1946). The operated larvae, which were tested for their humidity reactions before the operation, were then transferred to the breeding medium and allowed 12 hr. to recover. When these larvae were tested in the alternative chamber, they still showed the striking avoidance of the drier side with nearly the same reaction intensity they showed before the operation (Fig. 5A).

Other probable sites of the humidity receptors were then looked for. Among these are the anterior and posterior spiracles. Larvae in which either the anterior or posterior spiracles, or both, were covered (with cellulose paint) also showed marked avoidance of the drier side. Then the whole thoracic and abdominal surface was covered with vaseline. When such treated larvae were tested, it soon became clear that the intensity of reaction was greatly diminished, and out of thirty larvae twenty-six were almost indifferent on crossing from the moist to the dry side of the arena (Fig. 5B). Having obtained this result repeatedly, the skin of the larva was carefully examined. The skin was cut along the sides of the body, thus separating dorsal and ventral halves. The underlying tissues were scraped off until the skin became nearly transparent, was then mounted in glycerine, and examined under high magnification and very strong illumination. Two types of sense organs (Fig. 6) were found, both described briefly by Keilin (1915). The first type occurs in all the thoracic and abdominal segments. In each segment there are about thirty sense organs divided between the dorsal and the ventral surfaces. Each organ is in the form of a minute brown circular structure about 10μ in diameter. In section, it consists of a relatively thin, somewhat dome-like cuticular covering to the inner surface of which is attached a nerve fibre terminating in a sense rod. The nerve fibre is continued to the sense cells below.

The second type of sensory organ occurs only on the ventral surface of the thoracic segments, one pair in each segment. The two organs are very near to each other in the prothoracic segment and wide apart in the meso- and meta-thoracic segments. Each of these organs consists of three minute hairs arising from the floor of a slight depression of the cuticle. Below these hairs is a small oval cavity through which extends a somewhat deeply staining rod attached distally to the sensory bristles and proximally to a thread leading to the sense cells.

The possibility that the circular organs or tufted sense organs may act as hygro-receptors was tested by smearing various parts of the body with vaseline and then studying the reactions of the larva in the alternative chamber. The results obtained have shown that the larva was able to respond to the humidity difference offered, and avoided the drier side when any of the following parts of its body was covered, each in one experiment: (*a*) the whole of the dorsal body surface (except the head); (*b*) the whole of the abdominal surface; (*c*) the whole of the abdominal surface + the dorsal surface of the thorax. On the other hand, when the ventral surface of the thorax was carefully covered with vaseline or better with a thin coat of cellulose paint, the humidity reaction of the larva was greatly diminished or entirely eliminated.

The disappearance of the response of the larva to humidity when the ventral surface of the thorax is covered, and its appearance again when this part is exposed suggest that the tufted thoracic sense organs may be sensitive to humidity and may function hygroscopically (Pielou, 1940). To this may be added that larvae have often been observed on encountering the dry half of the arena, to stop for a few seconds at the humidity boundary, raise the anterior part of the body with the ventral surface facing the dry air, swing it from side to side and then

recoil to the moister half. It may be noted that these thoracic sense organs bear a striking structural resemblance to the 'tuft organs' described by Wigglesworth (1941) as the humidity receptors of the louse.

As regards the circular sense organs found all over the thorax and abdomen, it may be pointed out that their structure which somewhat resembles that of campaniform sensilla suggests that they may function as proprioceptive organs sensitive to mechanical stimuli (Wigglesworth, 1939).

The identity of particular humidity receptors has been established in a very few insects. In the mealworm, *Tenebrio* (Pielou, 1940), the pit peg and peg organs on the antennae are hygro-receptive, and in *Pediculus* (Wigglesworth, 1941) the 'tuft organs' on the antennae are sensitive to humidity. On the other hand, in mosquitoes (Thomson, 1938), in locusts (Kennedy, 1937), *Blatta orientalis* (Gunn & Cosway, 1938), and wireworms (Lees, 1943), the humidity receptors were not located and no experimental evidence on their form or structure was brought forward. Necheles (1925) merely suggested that the antennal hairs of the mosquitoes might be sensitive to humidity. In wireworms the results obtained by Lees (1943) suggest that the humidity reaction is initiated by the evaporation of water ('evaporimeter' receptor) from the head of the insect.

(4) *Mechanism of orientation*

The foregoing experiments demonstrated that the house-fly larva avoids dry air and prefers moist air. An attempt was made to find by what mechanisms this result is achieved.

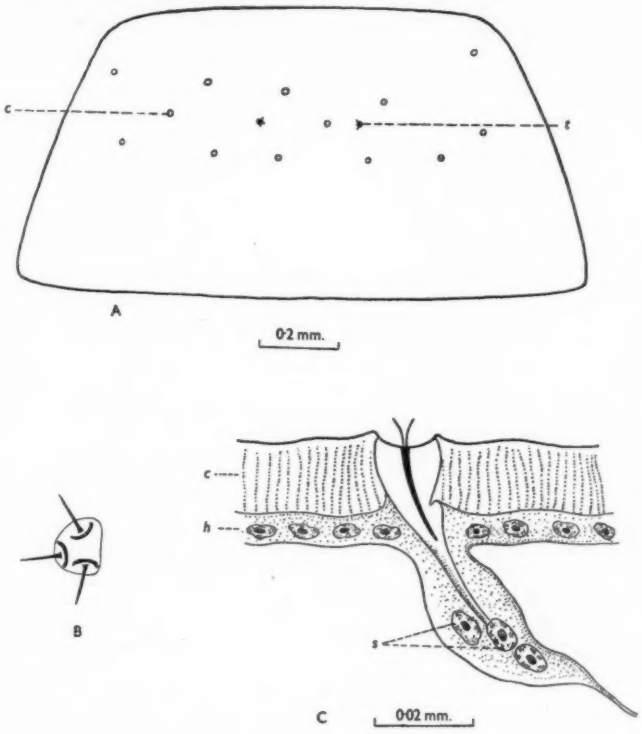


Fig. 6. A, mesothorax of larva (ventral view) showing the circular (*c*) and tufted (*t*) sense organs; B, tufted sense organ magnified; C, section of the same. *c*, cuticle; *h*, hypodermis; *s*, sense cells.

When a larva is exposed to a pair of low and high humidities in the alternative chamber, and crosses from the moist zone to the dry, its rate of movement instantly increases. Ultimately, however, the larva is brought back to the moist zone, where its original rate of movement is slowly restored. This simple mechanism of orientation, in which the rate of random movement depends on the intensity of stimulation, is termed orthokinesis (Gunn, Kennedy & Pielou, 1937).

Orthokinesis was also clear at uniform humidities.

For instance, the rate of movement of the larva at 33 % R.H. was nearly twice what it was at 98 % R.H. At the lower humidity the movement was somewhat agitated.

Another mechanism of orientation seemed at work when the larva crossed the humidity gradient from the moist zone to the dry. From an inspection of the tracks of the larva (Fig. 7) it is obvious that on

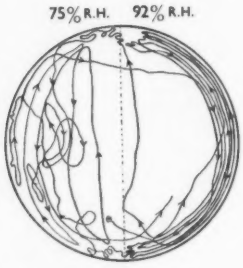


Fig. 7. Track of larva showing a klinokinetic reaction and sensory adaptation.

entering the dry side the larva no longer moves straightforwardly round the periphery of the arena, but begins to turn and follow a more irregular course. This is an example of klinokinesis (Gunn *et al.* 1937), a type of orientation mechanism in which the frequency of random turning or the rate of change of direction depends on the intensity of stimulation. The larva keeps turning on the dry side, until ultimately it crosses the boundary again to the moist side where it ceases to turn and moves straight.

There is a further mechanism of orientation which comes into action when the larva encounters a steep gradient as in an arena with the alternatives 62 or 95 % R.H. On reaching the humidity boundary the larva immediately turns and recoils backwards into the moist side. This type of mechanism seems to be a directed one (taxis). The tracks of the larva shown in Fig. 2 give a typical picture.

This orientation mechanism may be either klinotactic (i.e. involving a comparison of the intensity of stimulation in successive intervals of time), or tropotactic (i.e. involving a comparison of the stimulus simultaneously by means of bilaterally placed sense organs). During its movement the larva regularly moves the anterior part of its body right and left. These alternate lateral deviations are those by which the larva 'compares' between two light intensities in orientating itself to light klinotactically (Fraenkel & Gunn, 1940) and may serve the same purpose with regard to humidity, the humidity receptors are, in fact, arranged symmetrically on each side of the thorax. But they are ventral so that they seem incapable of mediating a tropotaxis. They are, on the other hand, far enough forward to partake in the lateral deviations necessary for klinotaxis. This klinotactic reaction is seen to the best advantage when the larva approaches the humidity boundary obliquely or nearly parallel to it.

House-fly larvae also show sensory adaptation to adverse humidity. The importance of this phenomenon was emphasized by Wigglesworth (1941) as a factor in the behaviour of the louse. Fig. 7 shows that the course of the larva on the dry side is more convoluted at first, but becomes gradually less convoluted, increasing the likelihood that the larva will pass over again into the moist side. Without this adaptation, the larva might become trapped in the adverse zone.

It seems that at least three mechanisms (orthokinesis, klinokinesis and klinotaxis) contribute to the general humidity behaviour of the larva in the alternative chamber. The exact part played by each mechanism may be difficult to define, but it seems evident that the sharp avoidance of the lower boundary is mainly a matter of klinotaxis. This mechanism is most effective in keeping the larva away from the dry side. The increased frequency of turning (klinokinesis), together with the adaptive ability of the larva and the change of the rate of movement (orthokinesis) on the dry side, are also important factors in the humidity behaviour of the larva, and their combined effect eventually brings the larva back to the moist side. Sometimes all these mechanisms are visible in the behaviour of the same larva.

These results show, however, that there is no distinction in kind between klinokinesis and klinotaxis. In its extreme form, produced merely by a change in the stimulation pattern, klinokinesis becomes klinotaxis.

Experiments in the alternative chamber have already shown that in the low humidity range (below 50 % R.H.), no significant reaction was obtained to differences as large as 25 or 30 % R.H., i.e. none of the above mechanisms seems to operate. Similar behaviour in wireworms was accounted for by Lees (1943) as follows. The clear avoiding mechanisms in the higher humidity range are virtually eliminated in the lower humidity range by the intensely active larva being carried over the humidity boundary into the lower humidity in the opposite side. The behaviour of the house-fly larva at the lower humidity range could be explained also along these lines.

Kineses and taxes have been reported as humidity orientation mechanisms in a number of insects. Orthokinesis has been established in the locust (Kennedy, 1937). With wireworms (Lees, 1943) orthokinesis and klinotaxis are the mechanisms involved. In the louse (Wigglesworth, 1941), orientation takes place klinokinetically and klinotactically. In *Tenebrio* all three mechanisms have been reported (Gunn & Pielou, 1940).

IV. TEMPERATURE REACTIONS

(1) *Methods*

The arena was here enclosed by a metal ring. Half of the arena rested on one tank, and half on the other. About 1 mm. below the floor were blotting-papers saturated with water or salt solutions to control the humidity. Unless otherwise stated, the experiments were carried out at about 100 % R.H. The arena was provided with a double roof formed by two glass plates separated by plasticine round the edges, so as to leave an insulating air layer of about 10 mm. between them. The junction between the roof and the metal ring was rendered air tight with vaseline. In the middle of each roof-plate was a small circular hole which could be covered with a vaselined microscopic slide.

When temperature* and humidity equilibrium had become established, a larva was introduced through the circular hole which was then covered again. A few minutes afterwards the behaviour of the larva was observed. Since the space between the floor of the alternative chamber and the roof was narrow, there was no appreciable vertical gradient of temperature which might affect the behaviour of the larva.

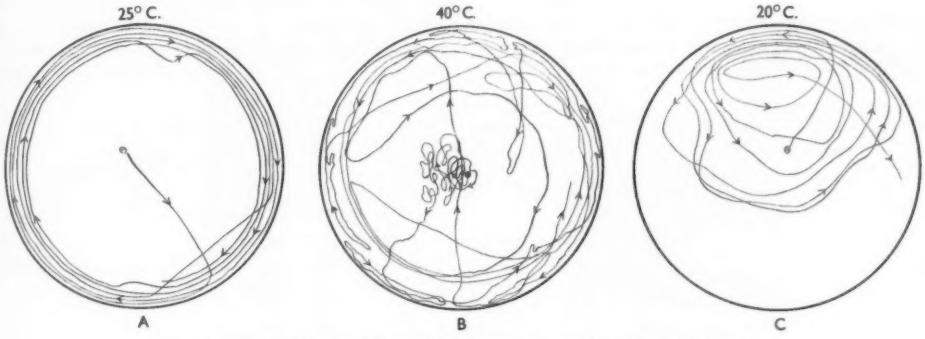


Fig. 8A, B, C. Tracks followed by larvae at uniform temperatures.

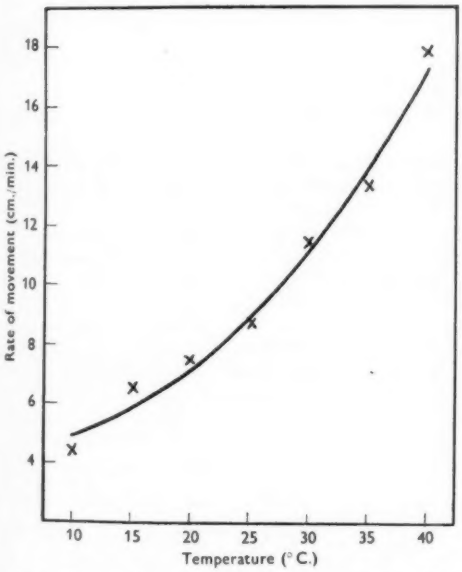


Fig. 9. The relation between temperature (from 10 to 40° C.) and the rate of movement of the larvae.

(2) *Results*

(a) *Uniform temperatures*

The following uniform temperatures were used, each in one experiment: 10, 15, 20, 25, 30, 35 and 40° C. The courses of the larvae (Fig. 8A) were almost straight at medium temperatures (e.g. 20, 25, 30° C.), less so at 35 and 10° C. and convoluted at high temperatures (e.g. 40° C.) (Fig. 8B). A few larvae showed a bias and moved spirally making successive circles to one side (Fig. 8C).

Between 10 and 40° C., the rate of movement rose with temperature (Fig. 9). Thus at 10, 30 and 40° C., the average rate of movement (in ten experiments each lasting 10 min. at each temperature) was 4·3, 11·1 and 17·6 cm./min. respectively (Table 1).

In most of the experiments with high temperature (e.g. 40° C.), the larvae moved rapidly initially, but later slowed down and pursued less convoluted or even straight courses (Fig. 8B). In fact, they became gradually adapted to this high temperature.

* The temperature of the floor was measured by a 'skin temperature recorder', the sensitive junction of this instrument being inserted through the circular hole in the roof. The disturbed humidity equilibrium was restored shortly afterwards.

Table 1

Temperature (° C.)	No. of larvae used	Average distance travelled (cm.)	Average speed (cm./min.)
10	10	43	4·3
15	10	63	6·3
20	10	72	7·2
25	10	85	8·5
30	10	111	11·1
35	10	130	13·0
40	10	176	17·6

(*b*) *Alternative temperatures*

Preliminary experiments were again carried out with extreme alternatives. Fig. 10A shows a typical track of a larva given a choice of 25 or 40° C. The larva spent nearly all its time on the cooler side and strongly avoided the higher temperature. On approaching the mid-line from the cool side, larvae turned back but sometimes made their turns too late to regain the cool side at once. They then continued on the warmer side, moving rapidly and erratically on a convoluted course until they eventually regained the cooler zone (Fig. 10B). When larvae were offered the choice of 25 or 10° C., an avoidance of the lower temperature appeared (Fig. 10C), but it was not as strong as avoidance of the higher temperature (40° C.).

The reactions of larvae to other differences on the temperature scale (8–40° C.) were then studied in order to find how sensitive they were, and what were the threshold high and low temperatures necessary to elicit a response. In most cases the temperature on one side was kept at 25° C. and progressively lowered or raised on the other side. The temperature differences used varied from 1 to 30° C.

The results obtained showed that below 25° C. larvae were not sensitive to a temperature difference of 5 or 10° C. Thus, when offered the choice of 25 or 15° C., the larvae showed no preference. When the temperature on the cooler side was lowered to 13° C. a definite, though weak reaction was obtained. The reaction was strong at 25 or 10° C. (Fig. 10C), and stronger still at 25 or 8·5° C., when larvae coming from the warmer side, turned back into it at once or else followed a more or less convoluted course on the cooler side, where they became very sluggish.

Above 25° C. the larvae were again indifferent to a difference of 5 or 8° C, Thus they were indifferent, given choice of 25 or 30° C. or 25 or 33° C. At 25 or 34° C. (9° difference), however, the larvae showed slight avoidance of the higher temperature. The avoidance became strong with a choice of 25 or 37° C. and very strong with 25 or 39° C. When the temperature range used was entirely above 35° C., the larvae were sensitive to a difference of only 1° C., avoiding the higher temperature. Below 15° C., on the other hand, the larvae seemed much less sensitive. For instance, while there was a strong reaction to a difference of 1° at 35 or 36° and 36 or 37° C., there was none to the same difference at 13 or 12°; 12 or 11°; 11 or 10°; 10 or 9° C. It seems therefore that, under the given experimental conditions, there was a rapidly increasing sensitivity to temperature differences as the temperature approached 35° C. It also seems that there was a relatively wide temperature range extending from about 15 to 33° C. in which the larvae were generally indifferent, even to differences as great as 15° C. (e.g. at 15 or 30°, or 17 or 32° C.).

The above experiments were repeated at 50 % R.H. and in dry air. In both cases larvae reacted to a difference of 6° at 25 or 31° C. They avoided the warmer side, either turning away from it or following

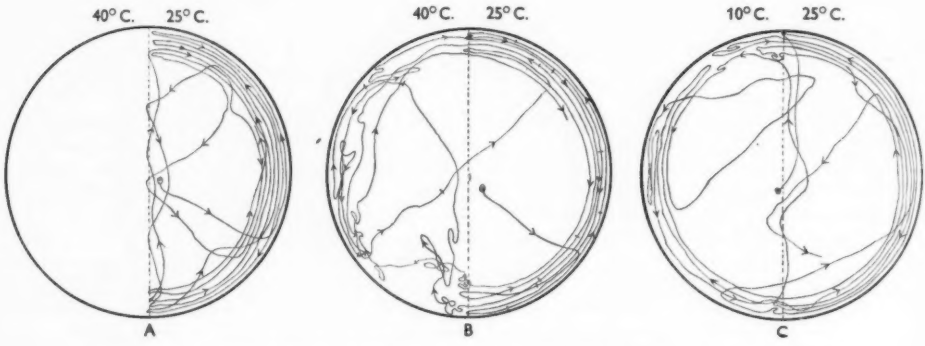


Fig. 10A, B, C. Tracks followed by larvae showing reactions to high and low temperatures. Sixty seconds are marked on the tracks in Fig. 10B.

an irregular course on it. The avoidance was stronger at 25 or 32° C. Bearing in mind the larvae were indifferent to the same difference at higher humidity, it seems that they prefer a lower temperature in dry air than in moist.

At the lower temperature range (below 25° C.) there was no appreciable change in the reactions of the larvae to temperature differences under various conditions of relative humidities (Table 2 and Fig. 11).

When these experiments were further repeated at about 100 % R.H. and with a moistened floor, so that the larvae were in direct contact with water as is usually the case in the natural habitat, it was found that they were indifferent to an even greater difference of temperature. Thus they showed no preference when offered the choice 25 or 39° C. With 25 or 40° C. only one larva out of ten avoided the higher temperature.

Comparing the results obtained in the alternative chamber in (*a*) dry air; (*b*) moist air; (*c*) moist air with water (Table 2 and Fig. 11), it seems that the range of temperature difference of the larvae (above 25° C.) was influenced by the relative humidity in the chamber and much more so by direct contact with water. The indifference range was higher in moist air with water or moist air alone than in dry air. In other words, the larva seemed to prefer a lower temperature in dry air, and did not avoid so high a temperature in moist air. This was also observed by Gunn & Cosway (1938) in the cockroach. The apparent preferred temperature of the individuals which react to humidity is higher in moist air than in dry. In their experiments it seemed that these individuals 'strike a kind of balance between their humidity reaction and their temperature reaction'. In our case, any reaction to humidity is excluded in the experiments where the relative humidity was 100 % R.H. on both sides. In the experiments using $Ca(NO_3)_2$ to produce 50 % R.H., the cooler side would be moister than the drier side by about 5 % R.H. in an alternative chamber of 25 or 30° C. (Adams & Merz, 1929). We know, however, from the work on the humidity reactions that the larva is quite indifferent to humidity differences in this part of the humidity range. Thus it seems that there can have been no interference by the humidity reactions in these experiments on temperature preference at different humidities. The humidity must, therefore, have

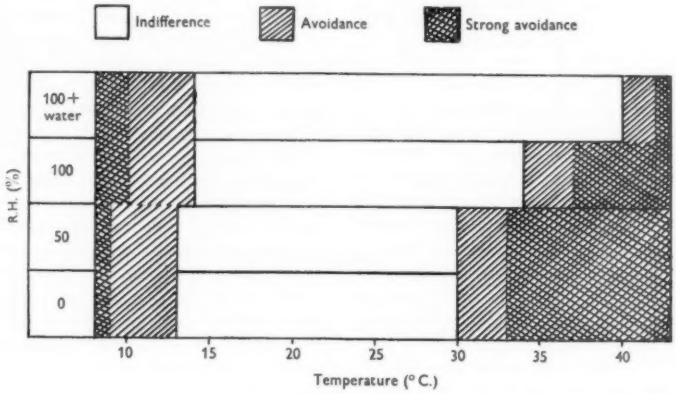


Fig. 11. Effect of air humidity and liquid water on avoidance by the larvae of temperature extremes in the alternative chamber.

Table 2

	Higher temperature			25° C.	Lower temperature	
Approx. R.H. (%)	Strong avoiding reaction at	First avoiding reaction at	Zone of indifference	Zone of indifference	First avoiding reaction at	Strong avoiding reaction at
100	37° C. or higher	34° C.	25–33° C.	25–15° C.	14° C.	10° C. (or under)
50	33° C. or higher	30–31° C.	25–30° C.	25–14° C.	13° C.	9–10° C. (or under)
0	33° C. or higher	30° C.	25–29° C. or 30° C.	25–14° C.	13° C.	9° C. (or under)
100+water	42° C. or higher	40° C.	25–39° C.	25–15° C.	14–13° C.	10° C. (or under)

acted through some physiological mechanism to change the preferred temperature at different humidities.

The striking indifference of the larvae to higher temperature (as high as 39 or 40° C.) when they are in direct contact with water may have some bearing on the results obtained by Thomsen & Thomsen (1937). According to these authors about 3 days' old larvae tested in a temperature gradient filled with horse-dung preferred a zone between 30 and 37° C. In one series of their experiments their figures indicate that about 16 % of the larvae are usually found at a temperature of 39·1° C. In another series, they have recorded 7·6 % of the larvae at 42·6° C.

(c) *Preconditioning*

The possible effect of preconditioning on the reactions of the larvae was also tested. Twenty larvae were divided into two groups of ten each: one group was kept at about 12° C., the other at about 35° C. for 5 hr. Both were then offered the choice of 25 or 12° C. and 25 or 35° C. respectively. In eighteen out of twenty, the larvae showed no adaptation towards the temperatures to which they were previously exposed, the larvae preferred the 25° C. in both cases. When offered the choice of 12 or 35° C., these larvae seemed to avoid both sides and a preference for the middle zone appeared and this was still more marked when the alternative temperatures used were 8·5° or 40° C. (Fig. 12).

(3) *Temperature receptors*

No chief site of temperature sense could be located in the larva. Larvae deprived of their oral lobes,* which are the seat of several sense organs (Bolwig, 1946), avoided higher (e.g. 37° C.) and lower (e.g. 10° C.) temperatures as they did in the intact state. It seemed that the sensitivity to temperature is distributed over the general body surface, which is richly provided with a complex network of nerve cells lying beneath the hypodermis (Viallanes, 1882).

No special temperature receptors have been identified in most of the insects whose temperature reactions have been studied. Wigglesworth & Gillett (1934) considered that the numerous finely pointed, relatively thick-walled trichoid sensilla of *Rhodnius* are probably temperature receptors; and Wigglesworth (1941) suggested that the peg organs with sharp tips on the fifth antennal segment of *Pediculus* may have a similar function, but the results obtained have shown that the temperature sense probably resides in the antennae as well as elsewhere.

* The oral lobes were tied with a very fine silk thread and then cut off with fine scissors-forceps. The larva was allowed to recover from the operation for 12 hr. in the breeding medium.

(4) *Mechanism of orientation*

The mechanisms of orientation of the larva to temperature are the same as those to humidity. Orthokinesis was demonstrated in both uniform and alternative temperatures (Figs. 9 and 10B). Larvae offered 20 or 32° C., although showing no avoidance of either side, moved at a mean rate of 8·2 cm./min. on the cool, and 12·4 cm./min. on the warm side. Also klinokinesis came into action as the larva crossed to the adverse side of the arena (Fig. 10B). On the other hand, when the larva encountered a steep temperature gradient, it showed what seemed to be klinotactic reaction (Fig. 10A). As to the relative contribution made by each type of mechanism to the general temperature behaviour of the larva, it seems that the marked avoidance of high temperatures in the alternative chamber is to a great extent brought about by the operation of klinotaxis. This mechanism is no doubt very effective in keeping the larva away from the adverse zone as it crosses the temperature boundary. Klinokinesis and orthokinesis, together with the gradual adaptation of the larva to high temperature, are also important factors in the orientation of the larva to temperature.

In reactions to lower temperatures, klinokinesis seems to be the main mechanism involved, although klinotaxis may come into action when the larva encounters a very steep gradient. There is no evidence of an orthokinetic protection against low temperature.

Thermo-klinokinesis and thermo-klinotaxis have been reported in *Pediculus* (Wigglesworth, 1941). The tracks published by Weber (1929) for *Haematopinus* and by Homp (1938) for *Pediculus* also seem to show thermo-klinokinetic reaction (Fraenkel & Gunn, 1940).

V. REACTIONS TO SMELL

(1) *Methods*

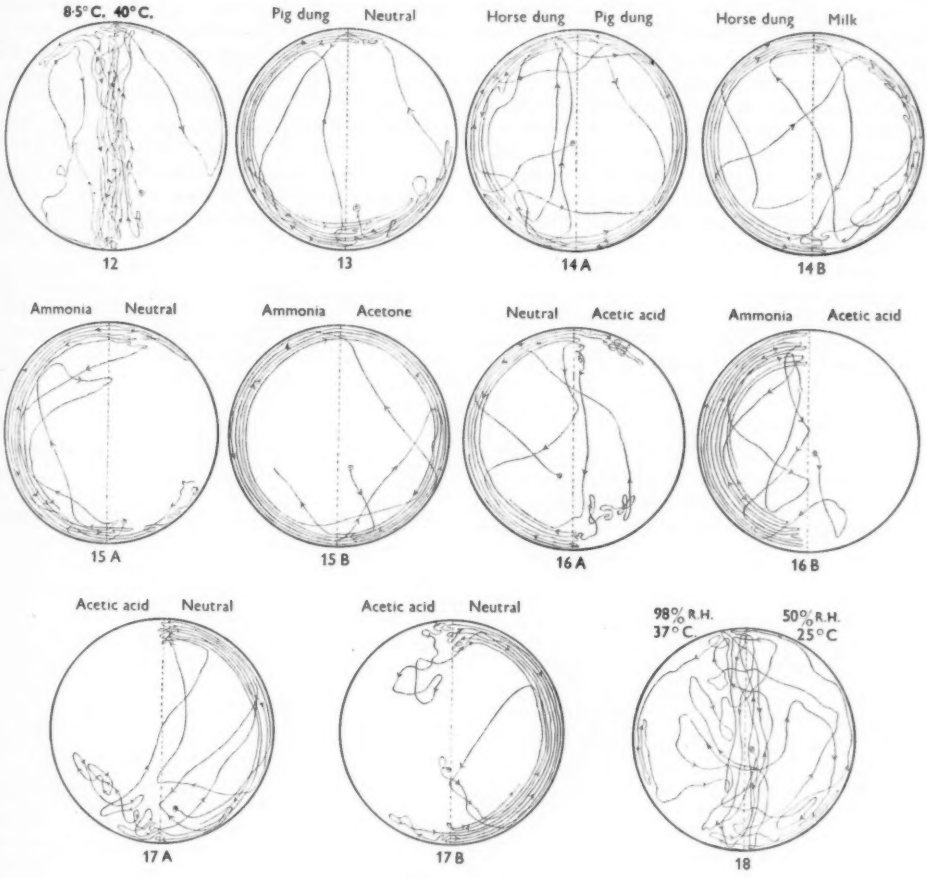
The method used for testing the reactions of the larva towards smell was roughly the same as that used in the humidity experiments. The arena was left open above. The blotting-papers were soaked in dilute solutions of whatever substances were to be tested as odours. The relative humidity in all experiments was near saturation (about 95 % R.H.). It was assumed that under such conditions the warm odours would be carried upwards by the convection currents and form in still air a steep gradient in the median zone of the arena (Wigglesworth, 1941). The following odours were used as they might be expected to form part of the normal environment of the larva (Bolwig, 1946): ammonia, acetic acid, acetone, phenol, ethylamine, trimethylamine, propionic acid, butyric acid and formic acid. All these substances, except the first two, were used at a concentration of 1 %

ammonia and acetic acid being diluted to 0·5%. The concentration of these solutions was kept constant throughout the experiments.

The reactions of the larvae were also tested towards some natural breeding substances such as diluted milk on which the larvae were previously fed, pig-dung and horse-dung. The dung was diluted and filtered and the blotting-papers saturated with the filtrate.

(2) *Results*

When one side of the arena was untreated, and the other contained pig-dung, horse-dung or milk, the larvae always strongly avoided the neutral side. The avoidance was strongest with pig-dung, the larvae returning to that side almost immediately after crossing into the neutral side (Fig. 13). The tracks of



Figs. 12–18.

Fig. 12. Tracks of larva given the choice of two temperature extremes.
Fig. 13. Reaction of larva to the smell of pig-dung.
Fig. 14A. Tracks of larva where half arena provided smell of horse-dung and half that of pig-dung; B, tracks of larva given the choice of the smell of horse-dung and that of milk.
Fig. 15A, B. Reactions of larvae to the smell of ammonia and acetone.
Fig. 16A. Reaction of larva to the smell of acetic acid; B, reaction of larva offered the choice of the smell of acetic acid and that of ammonia.
Fig. 17A, B. Reactions of larvae deprived of one antenna (left) in A and (right) in B.
Fig. 18. Reaction of larva to temperature and air humidity.

the larvae were usually straight on the scented side and convoluted on the neutral side.

When pig-dung and horse-dung were offered as alternatives, the odour of horse-dung was slightly avoided in favour of the pig-dung odour; but horse-dung odour was strongly preferred to milk odour. Fig. 14A, B shows the sample tracks of larvae given these alternatives.

Sample tracks of larvae offered ammonia and acetone are shown in Fig. 15A, B. Larvae preferred the ammonia- or acetone-scented side and avoided the neutral side. With ammonia on one side and acetone on the other, the larva was almost indifferent.

When one side was neutral and the other contained acetic, formic, propionic or butyric acids, the larvae avoided the acid-scented side. The response was strongest to the smell of acetic acid (Fig. 16A). There was almost complete avoidance of acetic acid offered as an alternative to ammonia, the larvae recoiling into the ammonia-scented side on approaching the boundary (Fig. 16B).

Substances such as ethylamine and trimethylamine which resemble ammonia in smell were also preferred by the larvae. On the other hand, the larva was almost indifferent to the smell of phenol.

These results were in agreement with those obtained by Bolwig (1946).

(3) *Mechanism of orientation*

Apart from klinokinesis (Fig. 15A), there seems to be a directed response as the larva encounters a very repellent smell (Fig. 16B). This orientation may be either klinotactic or tropotactic. The olfactory organs of the house-fly larva have been identified by Bolwig (1946) as the so-called antennae or dorsal organs which are symmetrically situated on the oral lobes. The right antennae of five larvae and the left antennae of five others were cut off with very fine scissors-forceps and the wound sealed with paraffin. If tropotaxis were the mechanism involved, the larvae would be expected to perform circus movements or, in other words, to turn always towards the injured side on approaching the mid-line or during its movement in the unfavourable zone.

The treated larvae were released into an arena neutral on one side and acetic-acid-scented on the other. There was no indication of circus movement or of turning always towards the injured side on crossing the boundary. In fact, turning was towards both the intact and operated sides (Fig. 17A, B). Despite the lack of one antenna the avoiding reaction toward the adverse side was always strong and always succeeded in bringing the larva back to the favourable side. It may be concluded that this orientation is not tropotactic but klinotactic, i.e. it involves the comparison of two intensities of stimulation at successive times. This is most evident during the characteristic alternate lateral deviations of the anterior end of the larva when it encounters the adverse side. Gradual adaptation to the adverse side was also observed here, and is, presumably, an essential part of the mechanism of klinokinetic avoidance of adverse smells as with humidity and temperature.

Bolwig (1946) is inclined to believe that klinokinesis with adaptation is the principal orientation mechanism involved. In his opinion, klinotaxis usually does come into action, as the swing of the larva when it encounters an unfavourable field of stimulation is not usually sufficient to bring its anterior end into the more favourable side. Hence it is unable to compare two intensities of stimulation at immediately successive moments. Under the experimental conditions described here larvae encountering a steep gradient (e.g. Fig. 16B), turned back instantly to the favourable side. In fact, the lateral deviation of the anterior end of the larva was usually sharp enough to keep it entirely out of the adverse zone. This was most evident when the larva approached the boundary obliquely or nearly parallel with it, and avoided entering the adverse side by a single swing of its anterior end. It should be made clear that there seems no difference in the kind of response in klinokinesis and klinotaxis. The transition of the former into the latter depends on the stimulation pattern or on the 'sensitivity' of the insect.

VI. REACTIONS TO COMBINATIONS OF FACTORS

The foregoing experiments dealt with the reactions of larvae towards one variable factor at a time. Such a study is necessary for a complete analysis of behaviour, yet these conditions do not usually occur in nature where several environmental factors vary at the same time. An attempt was therefore made to study, under laboratory conditions, the reactions of larvae to two or more variable factors at the same time, the factors used being temperature, humidity, smell and light. The main object of these experiments was to find to which factor the larvae reacted most strongly.

The apparatus used was essentially the same as that used in the previous experiments. The arena was always kept favourable (e.g. moist air) on one side, and unfavourable (e.g. dry air) on the other. Then an adverse stimulus (e.g. high temperature) was introduced into the favourable half, and a favourable stimulus (e.g. moderate temperature) introduced into the other previously unfavourable half. Experiments in which light was used were carried out in a dark room. One-half of the arena was exposed to bright light (655 m. candle) and the other to very dim light.* The bright light fell from

* In this dim light, it was just possible to follow the track of the larva.

a heat-screened lamp held vertically above the centre of the experimental field. The dim light was produced by covering the opposite half of the arena with a stout piece of black card. In the smell experiments 1 % acetone and 0·5 % acetic acid were used. 25° C., about 98 % R.H., acetone and dim light were used as favourable stimuli, whereas 37° C., about 50 % R.H., acetic acid and bright light were used as unfavourable stimuli. Factors other than those studied were kept as constant as possible during the experiment.

(1) *Two-factor experiments*

(*a*) *Temperature and humidity*

The arena was kept at 25° C. and 50 % R.H. on one side, and 37° C. and 98 % R.H. on the other. Fig. 18 shows a common behaviour pattern given these alternatives. The larva avoided both sides of the arena and showed a marked preference to the intermediate zone. If it did stray into either side, it followed an irregular course. When conditions on both sides were made more adverse, as by lowering the relative humidity to 33 % on the dry side and raising the temperature to 39° C. on the other, the larva became practically confined to the intermediate zone.

(*b*) *Temperature and light*

The arena was arranged in such a way that one half was thrown into bright light with a floor temperature of 25° C., and the other into dim light with a floor temperature of 37° C. Fig. 19A, B shows the behaviour of a larva given these alternatives. The larva avoided the warmer and dimmer side and preferred the cooler and brighter side. The reaction varied in intensity; some larvae stayed almost exclusively on the cooler and brighter side (Fig. 19A), while some frequently strayed into the warmer and dimmer side where they followed a convoluted course (Fig. 19B). On regaining the cooler and brighter side they turned once or twice apparently in response to the bright light, but soon moved straight ahead. In fact, larvae became quickly adapted to bright light. This quick adaptation to light explains why light reactions seem to have no appreciable influence on the reaction to temperature.

(*c*) *Temperature and smell*

Here, the temperature was again kept at 25° C. in one half and 37° C. in the other. The warmer side contained acetone and the cooler side acetic acid. Fig. 20 shows a common behaviour pattern in such an experiment. The larva almost always recoiled into the cooler side soon after crossing the boundary or after it has made short irregular excursions on the warmer side. But the klinokinetic reaction to acetic acid was also evident in the convolutions in the course of the larva on the acid-scented side. Judging the intensity of reaction by the time spent by the larva on each side, it was found that out of 135 min. (total time of ten experiments), 102 min. were spent on the cooler and acid-scented side and about 32 min. on the warmer and acetone-scented side. In other words, the larva seems to prefer a favourable temperature with an adverse smell to an adverse temperature with a favourable smell. The reaction to temperature is apparently stronger in this case, and seems to be little influenced by smell.

(*d*) *Humidity and light*

Fig. 21 gives a typical picture of the tracks of the larva when one half of the arena was dry (50 % R.H.) and dim and the other was moist (98 % R.H.) and bright. The larva avoided the former half and either immediately turned back at the boundary to the moist and bright side, or followed a short convoluted course on the dry and dim side. This behaviour is similar to that obtained in the second series of these

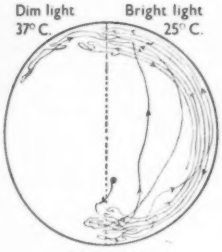


Fig. 19A.

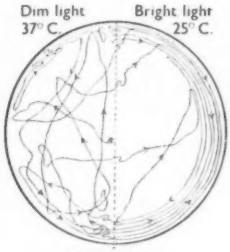


Fig. 19B.

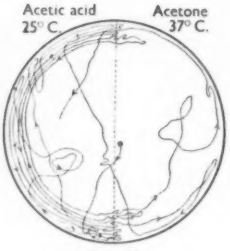


Fig. 20.

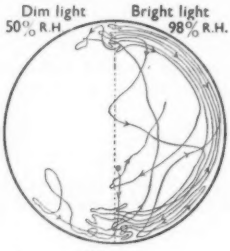


Fig. 21.

Fig. 19A, B. Reactions of larvae to temperature and light.
Fig. 20. Reaction of larva to temperature and smell.
Fig. 21. Reaction of larva to light and air humidity.

experiments (with temperature and light). The reaction to air humidity is here again not influenced by light, to which there is quick adaptation.

(*e*) *Humidity and smell*

The response of the larva to humidity and smell was first studied in a different apparatus (Fig. 22) which allowed of quick results. It consisted of two symmetrical halves. Each half comprised two large test-tubes with rubber stoppers, one held horizontally (T_1) and the other vertically (T_2). T_1 communicated with T_2 through a bent glass tube (T_3). Each vertical tube (T_2) also communicated with two bottles (B_1) or (B_2). The left and right outer bottles were joined by a long piece of rubber tubing (R) to a short T-shaped glass tube (T_4) connected to the compressed air supply. The two horizontal tubes (T_1) were joined by a larger T-shaped tube (T_5), the stem (S) of which was 2 cm. long. The vertical tubes were half filled, the right one with sulphuric acid and the left one with water. A piece of cotton-wool soaked in acetone was placed on a perforated zinc platform (P) above the sulphuric acid in one of the vertical tubes. A second piece of cotton wool, soaked in acetic acid, was placed over the water in the other tube. Thus, when the apparatus was working, the air reaching the tube (T_5) was dry and acetone-scented on the right side and moist and acid-scented on the left. Drying and moistening of the air were further ensured by its preliminary passage through bottles of sulphuric acid (B_1) and of water (B_2) respectively. Half way through experiments, the positions of the two vertical tubes were interchanged so as to eliminate any bias towards one particular side of the tube T_5. All the experiments were carried out in a constant temperature room at about 25° C. Before testing reactions to air humidity and smell together, three series of control experiments were carried out. In the first series plain air was passed through both sides of the apparatus. In the second series, dry air was passed in on the right side and moist air on the left. In the third series, the left air current was acetone-scented, and the right acid-scented. Table 3 gives the results of these experiments. Each series consisted of fifty trials with one larva each. These results gave no indication of a preference for one side over the other in the first series, nor did they show any deviation in the second and third series from what was expected from earlier work in still air.

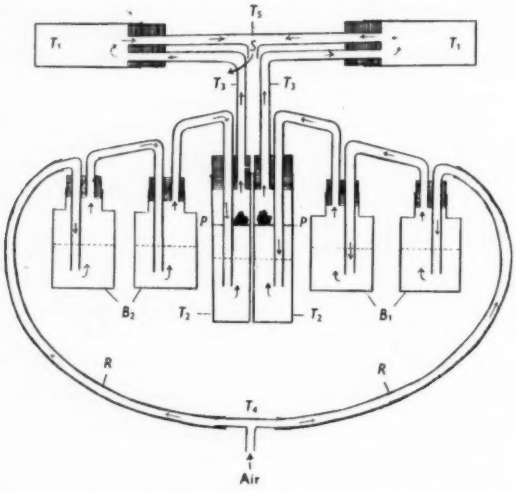


Fig. 22. Section of the apparatus for testing the response of larvae to smell and air humidity (modified from Bolwig). Arrows indicate the direction of air currents. For further explanation see text.

The apparatus, was then set up as previously described, and the reactions of the larvae to humidity and smell together were studied. Larvae were introduced one at a time into the short stem (S) of the T-tube (T_5). Out of fifty larvae, thirteen collected in the right horizontal tube (T_1) containing dry air and acetone scent, and thirty-seven in the opposite tube (T_1) containing moist air and acetic acid scent. Typical behaviour (Fig. 23) was for the introduced larva to turn immediately away from the acid-scented current and move up the acetone-scented though drier current. But, after making a meandering excursion in this direction, it turned back in the opposite direction. It meandered on until it en-

Table 3

First series. No. of larvae recovered from		Second series. No. of larvae recovered from		Third series. No. of larvae recovered from	
Left side	Right side	Drier side	Moister side	Acetone-scented side	Acid-scented side
23	27	9	41	39	11

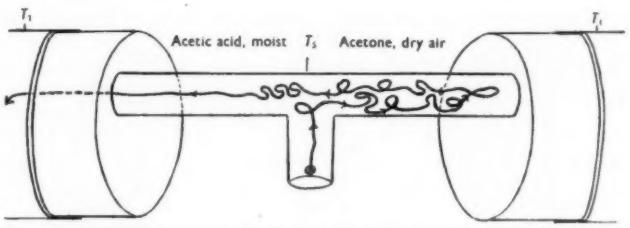


Fig. 23. A typical larval track in the tube T_5 of the apparatus shown in Fig. 22.

countered the moist acid-scented air. There, its track became temporarily more convoluted but soon straightened out and it went on until it fell into the left horizontal tube (T_1). Sometimes the larva did not reverse its direction quickly enough in the drier and acetone-scented tube and went on until it fell into the right tube (T_1).

The reactions of larvae to the same alternatives were also studied in the usual alternative chamber. Fig. 24 shows a typical result. The larva spent most of its time on the moister, acid-scented side and markedly avoided the opposite side.

In all these experiments humidity seemed to exert more control over the behaviour of the larva than did smell. The larvae preferred moister air even in the presence of an adverse smell, avoiding drier air when accompanied by a favourable smell.

(f) Light and smell

Fig. 25 shows the tracks of a larva in an alternative chamber offering the choice of a dim, acid-scented side and a bright, acetone-scented side. Larvae crossing the boundary into the acid-scented side either turned back at once or continued, on a convoluted course, into the acid-scented side. The reaction of the larva to smell was but little influenced by light to which the larva becomes more quickly adapted.

The results of the foregoing experiments in which the reactions of larvae towards two variable factors were studied, may be summarized as follows:

(1) Larvae avoid a zone of a higher temperature (37° C.) or drier air (50 % R.H.) even if they prefer smell or light of that zone, and they stay in a zone of moderate temperature (25° C.) or moister air (98 % R.H.) even if the smell or light in that zone could be avoided by itself.

(2) Larvae avoid both zones if one contains dry air (50 % R.H.) and the other is at a high temperature (37° C.), and tend to crawl between them.

(3) Larvae also avoid a zone of adverse smell (acetic acid) even if they prefer the light there (dim light), and they stay in a zone of preferred smell (acetone) even if the light intensity (bright light) there is one they would avoid if other factors were equal.

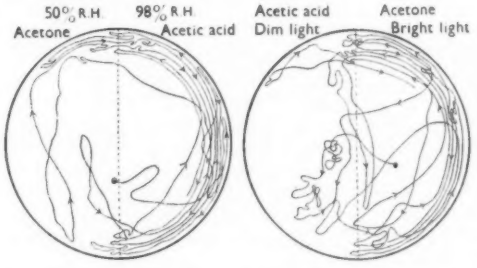


Fig. 24. Track of the larva in the alternative chamber showing reaction to smell and air humidity.
Fig. 25. Reaction of larva to smell and light.

It may be concluded from these results that: (*a*) light reactions have no influence on the reactions of larvae towards temperature, air humidity, or smell, (*b*) smell reactions have little or no influence on the reactions to temperature or air humidity, and (*c*) temperature and air humidity reactions greatly influence one another. A diagrammatic representation of these results is given in Fig. 26.

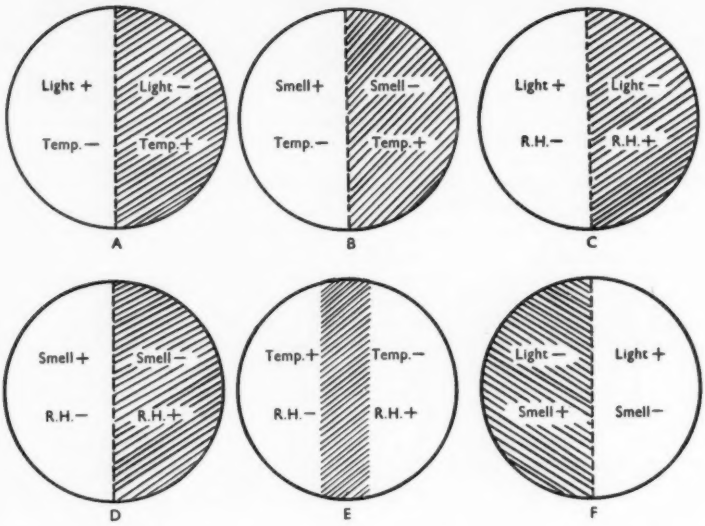


Fig. 26A–F. Diagrammatic representation summarizing the general behaviour of larvae given two variable factors. Shaded parts are the zones usually preferred by the larva. + = favourable; − = unfavourable.

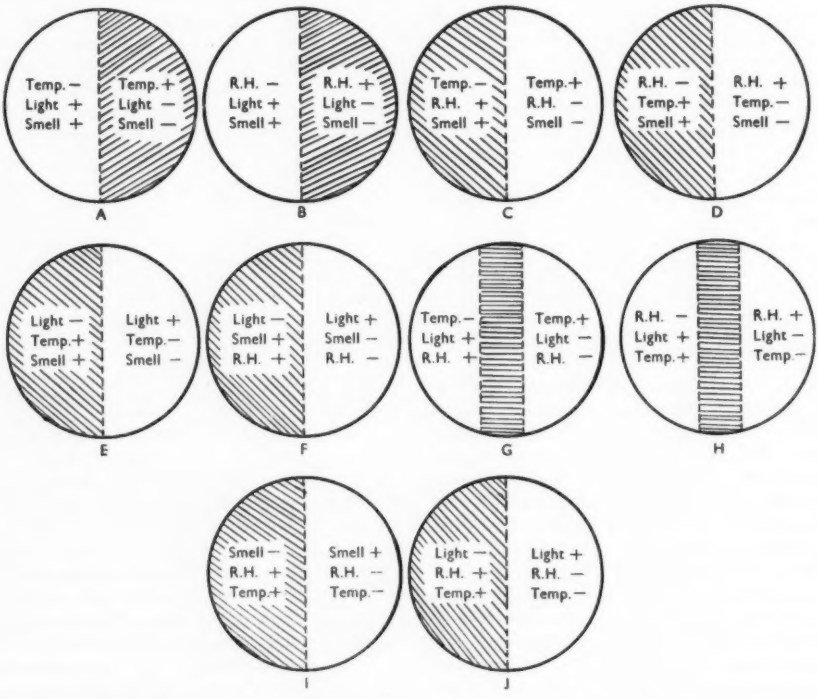


Fig. 27A–J. Diagrammatic representation summarizing the general behaviour of larvae given three variable factors. Shaded parts are the zones usually preferred by the larva. + = favourable; − = unfavourable.

(2) *Three-factor experiments*

The reactions of larvae towards three factors which varied at the same time were also tested. The results obtained were diagrammatically represented in Fig. 27 and support the conclusions from the two-factor experiments. The three-factor results may be summarized as follows:

(1) Larvae avoid a zone of higher temperature (37° C.) or drier air (50 % R.H.) even when either is accompanied by *both* a preferred smell and a preferred light intensity. They show a preference for a zone of moderate temperature (25° C.) or moister air (98 % R.H.) even when the smell and light intensity there would be avoided, other factors being equal (Fig. 27A, B).

(2) When one zone contains dry air (50 % R.H.) and the other is at a high temperature (37° C.), the larva seems to prefer the dry zone, if conditions on the other become more repellent, e.g. by the presence of an unfavourable smell (Fig. 27C–F). Light intensity differences introduce no change in the resultant behaviour (Fig. 27E–H).

(3) The larva sharply avoids a zone containing both high temperature and low air humidity even when the light or smell there are by themselves preferred. It stays in a zone of moderate temperature and high air humidity even when the light or smell there are by themselves avoided (Fig. 27I, J).

(3) *Four-factor experiments*

Experiments with four factors gave results exactly as were expected from the previous experiments. Fig. 28A–D shows typical tracks of four larvae.

The results obtained from all the previous experiments (with two, three or four factors varying at the same time), have clearly shown—under the present experimental conditions—that in a field of stimulation where temperature, air humidity, smell and light come into operation, the general behaviour of the larva is dominated by the first two factors. When these factors are such that they would be avoided by themselves, the larva becomes strongly reactive and persistently avoids their zones. On the other hand, light and smell have no appreciable influence on the reactions of the larva. This is probably due to its quick adaptation to smell and light, adaptation to light being the quicker and more pronounced. It should be made clear that the present picture of the reactions of the larva to these environmental factors is not complete, since each factor has been tested only over a limited range of intensities. The ranges were chosen with an eye to field conditions, but more experimental work is required before any generalization could be safely made.

VII. THE REACTIONS OF THE LARVA AND THE NORMAL ENVIRONMENT

The results obtained from the foregoing laboratory experiments have an obvious bearing on some of our present knowledge of the habits of the house-fly larva in its normal environment. The larva lives in decomposing organic matter such as dung, refuse, etc. Pig-dung and horse-dung are the most favourable breeding media (Thomsen & Hammer, 1936; Hafez, 1939). In a large heap of pig-dung or horse-dung, the distribution of the larvae is greatly influenced by the distribution of temperatures. In the centre of the heap where the temperature generated during the process of fermentation may be as high as 60–80° C., practically no larvae are found, while they predominate in the upper layer where the temperature ranges between 30 and 40° C. (Thomsen & Thomsen, 1937). According to these authors, the larvae are rarely found in regions where the temperature ranges between 40 and 45° C., but are often seen between 25 and 30° C. Thomsen & Thomsen also pointed out that the movement of the larva is erratic and agitated at these higher temperatures (40–45° C.). It dies at 47° C. after 15 min. and at 51° C. after 1 min. (Larsen, 1943). The avoidance by the larva of lower temperature (e.g. below 10° C.) may have

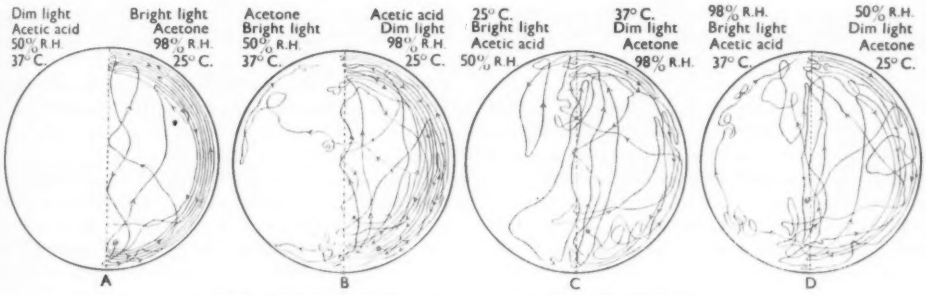


Fig. 28A–D. Reactions of larvae to four variable factors.

some ecological importance. In cold weather, larvae may migrate from the cold surface to the warm centre of the heap. But it should be pointed out that the temperature rarely falls below 10° C. at once in all parts of the breeding medium. If it does fall the larvae most likely become very sluggish and may fall into a state of torpor until the temperature rises again.

The striking preference of the larva for higher air humidities, and the fact that in the presence of saturated air all other humidities are avoided is not at all surprising as the larva lives in a micro-climate which is typically moist. The strong and consistent avoidance of low humidities may safeguard the larva against the danger of desiccation when some parts of the dung heap become dry. The larva would then migrate to moister parts. The resistance of the larva to desiccation is very low. For instance, when five larvae are kept in dry air (nearly 0 % R.H.) for 6 hr. at 27° C., they lose about 36 % of their original weight. This low resistance to desiccation has been made use of in controlling the larvae by spreading the dung into a thin layer and leaving it to dry (Lörincz & Makara, 1935; Thomsen, 1938; Hammer, 1941; Hafez, 1941).

The strong preference of the larva for the smell of pig-dung is in accordance with the results obtained from field observations and breeding experiments (Thomsen & Hammer, 1936). The preference for ammonia is consistent with the fact that fermenting manure heaps where this substance is produced, commonly teem with house-fly larvae.

The well-known negative phototaxis of the larva which has been recorded by several authors (e.g. Hewitt, 1914; Bolwig, 1946) explains the complete absence of larvae from the surface of the dung heap during the day and the quickness with which they withdraw and burrow downwards if any part of the heap becomes exposed.

The larva reacting with different strengths towards temperature, humidity, smell and light in a field of stimulation where all these factors vary at the same time is certainly found in nature. For instance, the weaker response to unfavourable light in the presence of a favourable smell (refer also to Fig. 26 F) has been observed in the field by Hammer (1941, p. 90) in the larvae of *Cryptolucilia* which '...at a distance of 4 to 5 cm. from the dung however moved towards it irrespective of the direction of light, which only means that at that distance the sense of smell is stronger than the sense of light'. As to the relative contribution made by these factors to the general behaviour of the larva in its normal environment, it is almost impossible to decide with any precision, but it is highly probable that in large manure heaps where the danger from desiccation hardly exists, temperature is the most important environmental factor influencing the behaviour of the larva. This was also emphasized by Thomsen & Thomsen (1937), who pointed out that the vertical distribution and movements of the larvae in natural conditions depend chiefly on their temperature preference and to a lesser degree on other factors such as light, humidity, chemical changes in the dung, etc. In small heaps, however, where the possibility of the dung's drying rapidly is greater, humidity may be a limiting environmental factor. On the other hand, smell and light are probably far less important environmental factors than temperature and humidity. The reason for this may be that the larva becomes more quickly adapted to the adverse conditions of light and smell which—unlike those of temperature and humidity—are not generally lethal.

It is possible to visualize the parts played by the various mechanisms of orientation demonstrated in the laboratory experiments, in the normal environment. House-fly eggs are laid on the surface of fresh dung, and after several hours these hatch into larvae which immediately burrow downwards in the dung and disappear from the surface. This may be due to a klinotactic response which leads the larva to a favourable smell and takes it away from unfavourable light. Other factors may also be involved. The gradual rise of temperature in the centre of the heap, causes the larvae to re-distribute themselves most probably in response to the operation of orthokinesis, klinokinesis and klinotaxis depending on the steepness of the gradient. These reactions lead the larvae into other sites of the dung heap where the temperature is optimal. The same may take place if conditions of humidity or smell become unfavourable. With regard to light, klinotaxis seems to be the main mechanism involved.

VIII. SUMMARY

The reactions of the house-fly larva (feeding stage) to humidity, temperature and smell and the orientation mechanisms involved have been studied in a circular arena divided into two halves. The reactions of the larva given various combinations of these factors and light have also been tested.

Reactions to humidity

1. The larva is least sensitive or is indifferent to humidity below 50 % R.H. Above this range, however, the larva avoids dry air; the intensity of avoidance increasing as the humidity range becomes higher. The larva is most sensitive within the range from 90 % R.H. to 100 % R.H., a difference of 5 % R.H. (at 95 or 100 % R.H.) is sufficient to ensure a complete avoidance of the drier side by nearly all individuals.

2. The larva generally shows no adaptation to the humidity to which it has been previously exposed.

3. The humidity behaviour of the larva is more closely correlated with the relative humidity and not with saturation deficiency. This suggests that the receptors act hygroscopically.

4. The probable humidity receptors are three pairs of sense organs lying on the ventral surface of the thorax, each organ bearing three minute hairs.

Reactions to temperature

1. Between 10 and 40° C. the rate of movement of the larva is directly proportional to temperature.

2. The larva strongly avoids 37° C. in saturated air and 33° C. in dry air. When the larva is in direct contact with water, it does not avoid 39 or 40°C. 10°C. (or under) is strikingly avoided; the larva is least responsive or indifferent to temperature differences between 25 and 30° C. in dry air, 25 and 33° C. in moist air, 25 and 39° C. in moist air with water and generally between 25 and 15° C.

3. The larva generally shows no adaptation towards the temperature to which it has been previously exposed.

4. There is no evidence of a localized temperature receptor in the larva. Decapitated larvae avoid high temperatures as strongly as normal larvae.

Reactions to smell

1. Pig-dung is preferred to horse-dung and the latter to milk.

2. The larva is attracted to the smell of ammonia, acetone, trimethylamine and ethylamine and repelled by the smell of acetic, formic, propionic and butyric acids.

Reactions to combinations of factors

The responses of the larva to twenty different combinations of temperature, humidity, smell and light have shown that under the present experimental conditions, the behaviour of the larva is dominated by temperature and humidity and least influenced by smell and light. The larva becomes quickly adapted to smell and light, adaptation to light being more pronounced and quicker.

Mechanisms of orientation

The orientation of the larva with respect to temperature, humidity and smell is mainly achieved by the operation of klinokinesis and klinotaxis. The former involves an increase in the frequency of random turning movements and is usually displayed as the larva enters a mildly unfavourable zone of stimulation; the latter is a mechanism of directed orientation involving the comparison of two intensities of stimulation at successive times and usually operates when the larva encounters a steep gradient between the favourable and adverse zones. In the response to low humidity or high temperature, a further mechanism involving an increase in the rate of movement (orthokinesis) of the larva comes into action. Sensory adaptation, evident in a steady decline of the increased rate of change of direction after prolonged exposure to the adverse condition, makes klinokinesis a mechanism of preference. By the operation of these mechanisms, the larva is usually kept out of zones of adverse stimulation.

The reactions of the larva to temperature, humidity, smell and light and their bearing on the habits of the larva in its normal environment are also discussed.

The author wishes to express his profound gratitude to Dr V. B. Wigglesworth, F.R.S., without whose valuable suggestions and kind help and guidance the present work would not have been possible. Sincere thanks are due to Professor H. C. Efflatoun Bey for his constant encouragement and to Dr John S. Kennedy and Dr A. D. Lees for their advice and painstaking criticism.

REFERENCES

ADAMS, J. R. & MERZ, A. R. (1929). Hygroscopicity of fertilizer materials and mixtures. *J. Industr. Engng Chem.* **21**, 305–6.

BENTLEY, E. W. (1944). The biology and behaviour of *Ptinus tectus* Boie (Coleoptera, Ptinidae). 5. Humidity reactions. *J. Exp. Biol.* **20**, 152–8.

BOLWIG, N. (1946). Senses and sense organs of the anterior end of the house-fly larvae. *Vidensk. Medd. naturh. Foren.* **109**, 82–217.

BUXTON, P. A. (1931). Measurement and control of atmospheric humidity in relation to entomological problems. *Bull. Ent. Res.* **22**, 431–47.

BUXTON, P. A. & MELLANBY, K. (1934). The measurement and control of humidity. *Bull. Ent. Res.* **25**, 171–5.

CROZIER, W. J. (1925–28). The orientation of animals by opposed beams of light. *J. Gen. Physiol.* **8**, 671–84.

DEAL, J. M. (1941). The temperature preferendum of certain insects. *J. anim. Ecol.* **10**, 233–356.

ELLSWORTH, J. K. (1933). The photoreceptive organs of a flesh fly larva, *Lucilia sericata* Meigen. *Ann. Ent. Soc. Amer.* **26**, 203–14.

FRAENKEL, G. S. & GUNN, D. L. (1940). *The Orientation of Animals.* Oxford.

GUNN, D. L. (1937). The humidity reactions of the woodlouse, *Porcellio scaber* (Latrielle). *J. Exp. Biol.* **14**, 178–86.

GUNN, D. L. & COSWAY, C. A. (1938). The temperature and humidity relations of the cockroach. V. Humidity preference. *J. Exp. Biol.* **15**, 55–63.

GUNN, D. L., KENNEDY, J. S. & PIELOU, D. P. (1937). Classification of taxes and kineses. *Nature, Lond.*, **140**, 1064.

GUNN, D. L. & PIELOU, D. P. (1940). The humidity behaviour of the mealworm beetle *Tenebrio molitor* L. III. The mechanism of the reaction. *J. Exp. Biol.* **17**, 307–16.

HAFEZ, M. (1939). Some ecological observations on the insect fauna of dung. *Bull. Soc. Fouad I Entom.* **23**, 241–83.

HAFEZ, M. (1941). Investigations into the problem of fly control in Egypt. *Bull. Soc. Fouad I Entom.* **25**, 99–143.

HAFEZ, M. (1948). A simple method for breeding the house fly *Musca domestica* in the laboratory. *Bull. Ent. Res.* **39**, 385–6.

HAMMER, O. (1941). Biological and ecological investigations on flies associated with pasturing cattle and their excrement. *Vidensk. Medd. naturh. Foren. Kbh.* **105**, 5–257.

HARTWELL, R. A. (1924). A study of the olfactory sense of termites. *Ann. Ent. Soc. Amer.* **17**, 131–62.

HERMS, W. B. (1911). The photic reactions of Sarcophagid flies. *J. Exp. Zool.* **10**, 127–226.

HEWITT, C. G. (1914). *The house fly* (Musca domestica *L.*) Cambridge.

HOLMES, S. J. (1905). The selection of random movements as a factor in phototaxis. *J. Comp. Neurol.* **15**, 98, 112.

KEILIN, D. (1915). Recherches sur les larves de Diptères cyclorrhaphes. *Bull. sci. Fr. Belg.* **49**, 15–198.

KENNEDY, J. S. (1937). The humidity reactions of the African migratory locust, *Locusta migratoria migratorioides* R. & F., Gregarious phase. *J. Exp. Biol.* **14**, 187–197.

LARSEN, E. B. (1943). Problems of heat death and heat injury—Experiments on some species of Diptera. *D. K. danske vidensk. Selsk. Biol. Medd.* **19**, 1–52.

LEES, A. D. (1943). On the behaviour of wireworms of the genus *Agriotes* Esch. (Coleoptera-Elateridae). I. Reactions to humidity. *J. Exp. Biol.* **20**, 43–53.

LEES, A. D. (1948). The sensory physiology of the sheep tick, *Ixodes ricinus* L. *J. Exp. Biol.* **25**, 145–207.

LOEB, J. (1890). *Der Heliotropismus der Thiere und seine Übereinstimmung mit dem Heliotropismus der Pflanzen.* Würzburg.

LOEB, J. (1905). *Studies in General Physiology*, 2 vols. Chicago.

LÖRINCZ, F. & MAKARA, G. (1935). Observations and experiments on fly control and the biology of the house-fly. *League Nat. Health Org. C.H. Hyg. rur. E.H.* 5.

LOWNE, B. T. (1890–2). *The blow-fly.* Vol. I. London.

MAST, S. O. (1911). *Light and the Behaviour of organisms.* New York and London.

MILLER, D. F. (1929). Determining the effects of change in temperature upon the locomotor movements of fly larvae. *J. Exp. Zool.* **52**, 293–313.

MITCHELL, H. & CROZIER, W. J. (1927–8). Photic orientation by two point sources of light. *J. Gen. Physiol.* **11**, 563–83.

NECHELES, H. (1925). *Arch. Schiffs- u. Tropenhyg.* **29**, 288–91.

PATTEN, B. M. (1914). A quantitative determination of the orienting reactions of the blow fly larva, *Calliphora erythrocephala. J. Exp. Zool.* **17**, 213–80.

PIELOU, D. P. (1940). The humidity behaviour of the mealworm beetle *Tenebrio molitor* L. II. The humidity receptors. *J. Exp. Biol.* **17**, 295–306.

PIELOU, D. P. & GUNN, D. L. (1940). The humidity behaviour of the mealworm beetle *Tenebrio molitor* L. I. The reactions to differences in humidity. *J. Exp. Biol.* **17**, 286–94.

POUCHET, G. (1872). De l'influence de la lumière sur les larves de Diptères privées d'organes extérieurs de la vision. *Rev. Mag. Zool.* **23**, 110–17, 129–38, 183–6, 225–31, 261–4, 312–16.

SWEETMAN, H. L. (1933). Studies of chemical control of relative humidity in closed spaces. *Ecology*, **14**, 40–5.

THOMSEN, E. & THOMSEN, M. (1937). Über das Thermopræferendum der Larven einiger Fliegenarten. *Z. vergl. Physiol.* **24**, 343–80.

THOMSEN, M. (1938). *Stuefluen og Stikfluen.* Copenhagen.

THOMSEN, M. & HAMMER, O. (1936). The breeding media of some common flies. *Bull. Ent. Res.* **27**, 559–87.

THOMSON, R. C. M. (1938). The reactions of mosquitoes to temperature and humidity. *Bull. Ent. Res.* **29**, 125–40.

VIALLANES, M. H. (1882). Recherches sur l'histologie des insectes. *Ann. Sci. nat. Zool.* **14**, 1–348.

WELSH, J. H. (1937). The chemoreceptors of certain dipterous larvae. *Science*, **85**, 430–1.

WIGGLESWORTH, V. B. (1939). *Principles of Insect Physiology.* London: Methuen.

WIGGLESWORTH, V. B. (1941). The sensory physiology of the human louse *Pediculus humanus corporis* De Geer (Anoplura). *Parasitology*, **33**, 67–109.

WIGGLESWORTH, V. B. & GILLETT, J. D. (1934). The function of the antenna in *Rhodnius prolixus* (Hemiptera) and the mechanism of orientation to the host. *J. Exp. Biol.* **11**, 120–39.

WILSON, R. E. (1921). Humidity control by means of sulphuric acid solutions with critical compilation of vapour pressure data. *J. Industr. Engng Chem.* **13**, 326.

(*MS. received for publication* 9. VII. 1948.—Ed.)

OBSERVATIONS ON THE BEHAVIOUR OF LAND-LEECHES (GENUS *HAEMADIPSA*)

By F. M. G. STAMMERS, M.A., M.B.

(With 1 Figure in the Text)

CONTENTS

	PAGE
I. Introduction	237
II. Previous investigations	
(1) Effects induced by bites	238
(2) Prophylaxis	238
(3) The behaviour of land-leeches	239
III. Present investigation	
(1) Material	239
(2) Description of experiments	239
(*a*) Coagulation of the blood	239
(*b*) Repellents	240
(*c*) Effect of temperature	241
(*d*) Effect of illumination	242
(*e*) Attraction offered by skin	242
IV. Discussion and conclusions	243
V. Summary	245
References	246

I. INTRODUCTION

At the request of Major-General (now Sir Alexander) Biggam, an investigation was carried out from August to December 1944 in the department of Physiology and Pharmacology, St Bartholomew's Hospital, London, for the purpose of ascertaining what methods could be employed to arrest the flow of blood which results from the bites of land-leeches, and whether their bites might be wholly or largely prevented. Concurrently their reactions to various stimuli were studied, as it was felt that a knowledge of their behaviour would assist in the elucidation of this problem which had arisen during the course of the campaign in Burma. During the latter part of 1945 investigations were continued at the Royal Naval School of Tropical Hygiene, Colombo, and were supplemented by observation of leeches in their natural surroundings, as they were found to occur in south-eastern Ceylon in a number of districts which could be easily and frequently visited.

Land-leeches are found in India and Ceylon, in Burma, Malaya and the Islands of the East Indies, and in China, Japan and Madagascar. In these lands they are confined to districts which have a heavy rainfall and where there is some measure of protection from the direct rays of the sun, for moist conditions are necessary for their terrestrial way of life.

Moore (1932) described their particular distribution. 'Land-leeches are found by no means everywhere in the jungle, forest or grass land, but chiefly in local aggregations or colonies. Such areas of concentration may be only a few feet or yards in diameter or they may cover acres, especially in forest grazing lands. Elsewhere one may proceed often for several miles along a seemingly favourable jungle trail and meet with scarcely a leech.'

In Ceylon they may be found in ravines amongst the undergrowth of ferns and grasses and in the vicinity of streams that pass through wooded country. Here they may occur in great numbers. Thus at Ingiriya, 30 miles from Colombo, there are several coconut plantations which yielded fifty or more leeches from every square yard of ground, their numbers being such that the landowners had difficulty in collecting the nuts during the wet seasons.

A number of writers have described their encounters with land-leeches, and it would seem that the resulting inconvenience and harm have been considerable. Shipley (1915) stated: 'In fact, of the many visible plagues of tropical Asia and its Eastern Islands they are perhaps the worst. Yet few have recorded their dread doings, and those few have escaped credence'. He goes on to say: 'Dr Charles Hose, for many years Resident at Sarawak, has told me that on approaching the edges of woods in Borneo you can hear every leaf rustling, and this is due to the fact that the eager leech, perched on its posterior sucker on the edge of each leaf in the undergrowth, is swaying its body up and down, yearning with an "unutterable yearning", to get at the integument of man or some other mammal.'

Hooker (1854), in his Himalayan Journals, stated that 'between Singtam and Chakoong, leeches swarmed in incredible profusion in the streams and in the damp grass and among the bushes: they got in my hair, hung on my eyelids, and crawled up my legs, and down my back. I repeatedly took upwards of a hundred from my legs, where the small ones used to collect in clusters on my instep: the sores which they produced were not healed for five months afterwards, and I retain the scars to the present day.'

Speaking of Ceylon, Tennent (1859) gave a colour-

ful description: 'Such is their vigilance and instinct, that on the approach of a passer-by to a spot which they infest, they may be seen among the grass and fallen leaves on the edge of a native path, poised erect, and preparing for their attack on man and horse. On descrying their prey, they advance rapidly by semicircular strides, fixing one end firmly and arching the other forwards, till by successive advances they can lay hold of the traveller's foot, when they disengage themselves from the ground and ascend his dress in search of an aperture to enter. Their size is so insignificant, and the wound they make is so skilfully punctured, that both are generally imperceptible, and the first intimation of their onslaught is the trickling of the blood, or a chill feeling of the leech when it begins to hang heavily on the skin from being distended by its repast. Horses are driven wild by them, and stamp the ground in fury to shake them from their fetlocks, to which they hang in bloody tassels. The bare legs of the palankin bearers and coolies are a favourite resort; and, as their hands are too much engaged to be spared to pull them off, the leeches hang like bunches of grapes round their ankles; and I have seen the blood literally flowing over the edge of a European's shoe from their innumerable bites'. He goes on to quote Marshall and Davy, both of whom mention 'that during the march of the troops in the mountains, when the Kandyans were in rebellion, in 1818, the soldiers, and especially the Madras sepoys, with the pioneers and coolies, suffered so severely from this cause that numbers perished'.

It may be claimed that some writers have rendered a somewhat exaggerated account of the land-leech and that the number of their bites could be much reduced by a knowledge of the country, an attentive eye, and by not remaining for long in places which they infest. However, during the recent war with the Japanese in Burma, these conditions could not be fulfilled, for men lived not according to their own inclinations, but to military necessity imposed by the varying fortunes of the campaign. As for those who marched against the Kandyans, the land-leech became an unpleasant reality and added considerably to the discomforts of life. Not only is there an almost universal feeling of repugnance for them, but also there are certain undesirable effects consequent on their bites. Blood may flow for 30 min. or more from the wounds which they inflict. Unless the bites have been numerous the total loss of blood is not large, but it soils the clothes at a time when facilities for washing are not always available, and even though the mouth-parts of the leech have been gently removed there is more often than not irritation at the site of the bite. Scratching to allay this irritation increases the likelihood of secondary infection, the resulting abscesses and ulcers being both slow in healing and all too common in hot and humid climates. Miller (1946) remarked that 'If infection did set in it could become highly dangerous. There have been times when my back looked like a dozen smallpox vaccinations, all working at once.' Harding & Moore (1927) declared that no one had ascertained how far this complication 'is primary and how far the result of scratching or other later contamination'.

II. PREVIOUS INVESTIGATIONS

(1) *Effects induced by bites*

It has long been known that from the bodies of leeches of the species *Hirudo medicinalis* Linn., there can be extracted a substance which inhibits the clotting of blood. The name, hirudin, was given to this substance which was generally thought to be injected into the host during the act of biting, thus ensuring a continuous passage of blood into the body of the leech. The familiar oozing of blood after the removal of the leech was thought to be due to the continued presence of hirudin in the tissues.

This view has been challenged by Lindemann (1939), who claimed that hirudin is never injected into the wound by *H. medicinalis* Linn., but is added to the blood while the latter is passing through the pharynx. His experiments suggest that the substance injected is allied to histamine, and that the continued bleeding is the result of paralysis of the capillaries which, by failing to contract, prevent the formation of a firm thrombus plug. It seemed possible that the prolonged bleeding which also follows the bite of the land-leech might be due to the same cause.

(2) *Prophylaxis*

The majority of land-leeches in the active and hungry state are about an inch long and of the thickness of a match, a diameter which can be reduced when the necessity arises, for they are capable of penetrating finely woven stockings and the lace holes of boots and shoes. Neither leather nor cloth impedes their progress, which by rapid looping movements takes them to the skin area of their choice. Their movement over the skin is not felt, as may be witnessed by finding them on almost any part of the body when the clothes are removed. From time to time leech gaiters have been devised. These have consisted of thick material, tied above or below the knee and closely approximating the shoe below. A number are warded off by this means, but many are capable of ascending the outside surface of the gaiter and of the trousers until a way to the skin is found. Moreover, such garments are hot in wear, impede walking, and will not prevent that species which, according to Beccari (1904), waits in bushes and attaches itself directly to the neck and hands.

Repellents as a means of protection have been in use for many years, for Hakluyt (1598) stated that the 'Minorite Friar, Odoric of Portenau, writing in A.D. 1320, says that the gem finders who sought the jewels around Adam's Peak "take lemons which they peel, anointing themselves with the juice thereof, so that the leeches may not be able to hurt them"'. Haekel (1883) painted a ring of carbolic acid high around his hunting boots and found that leeches never crossed the treated area. Other writers have used snuff and oil. Bailey (1945) placed tobacco leaves in the folds of his puttees which had been soaked in tobacco water. He goes on to remark that 'leeches hate this, and no doubt this kept many off me. In spite of this, in two hours march I picked one hundred and fifty off my clothes.'

(3) *The behaviour of land-leeches*

The sensations of leeches have been studied by a number of investigators, who have, however, directed their attentions to species which spend their lives in water. Of these, the medicinal leech, *H. medicinalis* Linn., has received more attention than any other. There have been a few observations on land-leeches; namely, that they display interest and activity when human breath is blown over them, and a note by Bailey (1945) that they dislike crossing sand. Smythe (1930) was of the opinion that they are blind and attack by scent alone. Certainly there must be guiding stimuli which lead them to their hosts, for, as Tennent (1859) stated: 'The individuals in the rear of a party of travellers invariably fare worst, as the leeches, once warned of their approach, congregate with singular celerity.' Coulter (1933) was also impressed by the fact that they are forewarned: 'They seemed to possess an uncanny instinct for detecting the distant presence of living creatures.'

III. PRESENT INVESTIGATION

(1) *Material*

All the leeches used in these experiments were of the species *Haemadipsa zeylanica* (Moquin-Tandon). A number were collected from the Burmese-Indian border and were flown to England in a Royal Air Force aeroplane. Others came from the districts around Ratnapura in Ceylon.

Upon receipt of the collection, the leeches were placed in glass cylinders of 8 in. in diameter and 10 in. deep. Each cylinder contained cut turf, moss and leaves. These were kept well moistened. The lid consisted of a piece of tightly tied cloth, the threads of which were close enough together and sufficiently strong to prevent the leeches escaping by penetration or severance. Such an arrangement was found suitable, for alertness and alacrity did not diminish over a period of several months. Initially it was thought that the severance of threads might be possible, for Green (1907), in a note on the boring power of land-leeches, described how he imprisoned one, which was full grown, in an entomological glass-topped cardbox, to find later that it had escaped through a hole perforating the cardboard, the excised portion having been reduced to powder.

(2) *Description of experiments*

(*a*) *Coagulation of the blood*

The flow of blood which results from the bites of land-leeches may continue for half an hour or more, at the end of which time it tends to slow or stop provided that the surface clot is not disturbed. In practice it was found that the clot remained only on parts of the skin that were uncovered, such as the forearms, but that the rubbing of clothes almost invariably resulted in the removal of the clot and the re-establishment of bleeding for another 15–30 min. To throw light on the cause of this continued bleeding the following experiment was devised.

A large-sized leech was placed on the forearm, and allowed to feed for 10 min. When other leeches were employed subsequently the time of 10 min. was adhered to so as to obtain uniform comparisons. At the end of this period, a drop of a saturated solution of sodium chloride was placed on to the body of the leech by means of a glass rod. Such an application invariably caused the leech to cease feeding and to raise its head. The leech was then quickly removed from the skin, and as soon as a fair-sized drop of blood appeared 6–8 small-bore capillary tubes were filled, sealed, and placed in water at a temperature of 37° C. At prearranged times, which were usually at half-minute intervals, the tubes were one by one unsealed, and their contents blown into water, the time being noted when the contents of a tube first came out as a uniform clot. This time was taken as the coagulation time of the blood, the average of which from five separate bites was 12·9 min. Following the bites of other leeches, the blood was allowed to flow freely away from the wounds for 2, 4, 6, 8, 10, 12, 20 and 30 min., that which had collected during this time being wiped away, the ensuing drop being taken up into capillary tubes for coagulation time estimations (Fig. 1.) The blood taken 2 min. after the removal of leeches had an average coagulation time of 7·8 min. That taken after 4 min. had an average coagulation time of 5·7 min. That taken after 6 min. had an average coagulation time of 3·9 min., and after 8 min. 3·2 min. At 10, 12, 20 and 30 min. the time was 3 min. The capillary tubes were as uniform in diameter as possible and gave the coagulation time of normal blood as being 3 min. Thus normality was

reached after 8 min. The method is the same as that described by Wright (1921), with modifications in that he used a single tube of a larger diameter.

A leech was placed on the skin and was left undisturbed until the sucking phase had become well established. Its neck was then cut through with a pair of sharp scissors and its body removed. In spite of this severe mutilation, the head and adjoining segments remained firmly attached to the skin and continued sucking blood. The blood welled up through the pharynx, with a rhythmic pumping movement which occurred initially about a hundred times a minute. At the end of 1 min. this rate had dropped to 80, and at the end of 3 min. to 40; thereafter the rate fell further, and ceased after a variable time, between 10 and 20 min. While the blood was still being pumped up, capillary tubes were filled by placing them into the cut pharynx. This blood remained incoagulable for days.

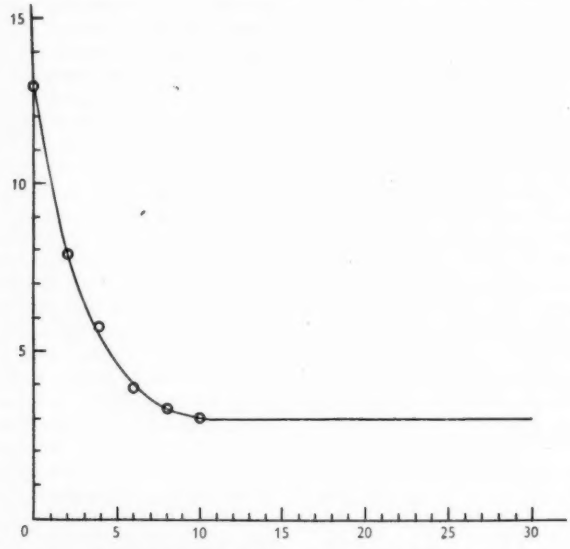


Fig. 1. Average coagulation time of blood taken at stated intervals after the removal of leeches from the skin.

(*b*) *Repellents*

A search was made for a substance which would prove so distasteful to leeches when applied to the skin or clothing that bites would be totally prevented. It was found early on that a certain number of substances possessed this desired quality. In order to obtain a measure of their comparative efficiency, increasing and known dilutions were prepared and tested against leeches until that dilution was reached at which repellency could no longer be demonstrated. Little difficulty was experienced in making such a differentiation. Arachis oil was found to be a suitable dilutant. It mixed with almost all the substances tested and possessed no power to repel. Where mixing did not occur, water was found to be a suitable alternative. The solutions were spread over pieces of fine filter-paper. Each paper measured 2 by 1 in. and received 20 cu.mm. of the solution to the square inch. The papers were then laid aside for 3 hr., and at the end of this time were tested separately, each being placed in the path of a leech so that the latter came in contact with it. If the substance, in the dilution under examination, was without repellent power, the paper was crossed without hesitation; a repellent effect being indicated by a quick withdrawal of the anterior sucker, followed by a change of direction which enabled the paper to be avoided. Contact might be made two or three times before the way round was found. Each paper was tested with ten leeches, the substance in question being considered a repellent in the dilution used if none of the leeches would cross the paper. On occasion a leech was presented with the same test paper two or three times, but this number was not exceeded for the reason that a sense of fatigue or accommodation was shown on repeated contact. Although not investigated, this effect seemed more apparent with some substances than with others, but may have been due to dehydration, since leeches were found to lose

weight under the conditions of the experiments, which were conducted at a relative humidity of about 75% and a temperature of 21° C. At the end of 6 hr. the treated papers were presented again and once more at the end of 24 hr., so as to obtain an estimate of persistency. Those substances that were promising were tested on the skin of man, the same quantity, namely, 20 cu.mm. of solution, being spread over each square inch of skin. Effectiveness as a repellent was indicated by a failure to cross the treated area and a refusal to bite.

Altogether seventy-eight substances were investigated on pieces of filter-paper. One, namely hydroxycitronellal, stood out as markedly superior to all others, for 1 part in a hundred of arachis oil was sufficient to repel leeches, no other substance being effective at this low concentration. Next in order of effectiveness were five substances—dimethyl phthalate, diethyl phthalate, ethyl hexanediol, vanillin and nicotine. All these repelled at a concentration of 1 in 60. Less effective were the following eleven substances which repelled at a concentration of 1 in 30: citronellal acetate; dimethyl-hexahydrophthalate, dimethyl-4-tetrahydrophthalate, *cis-iso*-eugenol, methyl-benzyl maleate, methyl iso-propyl phthalate, oleum cassiae, β-phenoxy ethyl acetate, phenyl methyl maleate, triethyl citrate, tri-*iso*-propyl citrate.

All the above, with one or two exceptions, were effective at 3, 6 and 24 hr. after application. The exceptions were oleum cassiae and citronellal acetate, both of which failed to repel after 6 hr. Nicotine, although uncertain in its action, produced marked paralysis after several contacts and was unique in this respect.

The following sixty-one substances were either completely ineffective or else possessed but little power to repel.

cis-acetyl-*iso*-eugenol, amyl acetate, amyl butyrate, amyl salicylate, arachis oil, beeswax, benzene hexachloride, benzyl benzoate, *iso*-butyl phenyl acetate, *n*-butyl *iso*-propyl phthalate, cetyl-trimethyl ammonium bromide, *p*-chloro-ethyl ether of eugenol, cholesterol, citric acid, citronellal-methyl phthalate, coumarin, cyclohexanone, dibutyl phthalate, dichloro-diphenyl trichlorethane, diethyl cinnamate, diethyl adipate, dimethyl maleate, dimethyl thianthrene, diphenyl phthalate, di-*iso*-propyl adepate, ethyl benzoate, ethylene glycol dioleate, ethyl lactate, ethyl phenyl-acetate, ethyl *iso*-propyl phthalate, fixone, geranyl acetate, geranyl-methyl phthalate, guanidine nitrate, hexyl resorcinol, β-hydroxy-quinoline, indalone, ionone, lemon juice, linalyl acetate, methyl benzoate, methyl-*n*-butyl phthalate, methyl-cyclohexanyl-acetate, methyl-cyclohexanol, methyl-cyclohexanone, methyl-cyclohexyl methyl phthalate, methyl phenyl-acetate, methyl *o*-toluate, α-naphthol, oleum thymi alb., paraffin (hard and soft), potassium phthalate acid, di-*iso*-propyl phthalate, pyrethrum, safrole, sodium *iso*-butyrate, sodium citrate, sodium valerate, tributyl phthalate, tri-*n*-propyl citrate, wool alcohol.

D.D.T., which is used extensively as an insecticide, proved to be quite ineffective against leeches. A number of land-leeches were confined to a jar which contained a sprinkling of the substance. Although many particles adhered to their bodies, they were as lively after 1 month as another batch of land-leeches which had acted as controls.

(c) *Effect of temperature*

Two glass test-tubes were set up in the vertical position, 2 in. apart from one another, so that their lower ends just touched the surface of a horizontal glass plate near its centre. The direction of light was at right angles to a line joining the two tubes. One tube was filled with water warmed to about blood heat (37° C.), while the other tube was filled with water, the temperature of which was the same as that of the room in which the experiment was conducted (19° C.). Leeches were then liberated on to the glass plate, across which they travelled in all directions. As they reached the edge, they were placed back on to the glass plate. No preference was shown for either tube, for leeches passed within a quarter of an inch, or an eighth of an inch, and were not attracted. On the other hand, whenever actual contact was made with the warmed tube, interest became apparent at once, for the leech quickly climbed on to the tube, probed the surface several times, and then assumed the position that is shown when on the skin of man in the act of taking a blood meal: namely, a flattening of the anterior end and a narrowing of the succeeding segments, in appearance not unlike that of the hoof and fetlock of a horse. In this position the leech would remain immobile for 10–30 sec., at the end of which time the anterior sucker became detached from the tube and the gentle probing movements were resumed. Alternate probing and application were repeatedly observed, interest in the tube continuing for several minutes while the contained water remained warm. The same behaviour was observed when the experiment was repeated in Colombo, where the temperature and relative humidity approximate to those of the natural surroundings of the leeches. Here the water in the one tube was at room temperature (30° C.), whilst that in the other tube was warmed initially to 37° C. Leeches placed on the cooler of the two tubes never exhibited these movements but moved rapidly away.

The human breath acted as a stimulus to the leeches causing them to display great activity, for within a short time of such a stimulus they were to be seen ascending the grass and other vegetation in

which they had been resting. This behaviour was displayed by leeches living in their natural habitat in jungle country and by others which had been captured and confined. Their sensitivity seemed to be of a high order, for a response could be elicited from a distance of several feet, and by gently blowing through the cloth covers of their containers. The same activity was produced by atmospheric air which had been bubbled through water and warmed. When such a stream of air was passed through a glass tube so that the issuing air was directed on to a leech, the latter, no matter in which direction it happened to be proceeding at the time, invariably stopped, raised the anterior portion of its body into the air, and after several side to side swaying movements lowered the head again and turned towards the direction of the current. This it reached by quicker movements than were exhibited before receiving the stimulus. If the direction of the current of air was altered before the mouth of the glass tube was reached, the leech correspondingly altered course. When breath and atmospheric air were bubbled through a large volume of water of the same temperature as the room in which the leeches were housed, there was little or no movement, while dry air at all temperatures induced a movement away from the source.

(*d*) *Effect of illumination*

Leeches were confined to a glass jar, and were placed in a dark room, the temperature and humidity of which were kept constant and which was illuminated by two electric bulbs. Provided that the inside walls of the jar were kept well moistened and care was taken not to breathe in the vicinity of the leeches, the latter remained in a state of quiescence over long periods. If one of the lights was turned out, movements of extension and exploration began immediately, a state of activity which persisted from a few seconds to several minutes. If, when the leeches had become quiet again, the light was turned on, no movement could be detected. A repetition of the experiment by turning one of the lights off and on, provided results which were substantially the same, but with each succeeding decrease in illumination the response became less and less and at the end of the fifth or sixth trial ceased altogether. This induced state of fatigue, or accommodation, evidently operated only for a decrease of illumination, for, if at this stage a breath was passed over them, they once again became active, their exploratory movements showing that they had lost little or none of their former liveliness. When these had ceased, it was found that the stimulus of a decrease of illumination again had an effect. This result could be repeated several times. When the experiment was persisted in over a long period it was found that the activity induced by warm moist air eventually failed to occur, but that if the leeches were left undisturbed for an hour or more, they once again became receptive. On no occasion throughout the time that they were observed did an increase of illumination call forth a response.

An attempt was made to ascertain what would be the least reduction of illumination which would induce a response. Two 60 W. bulbs were chosen, which within the limitations of the grease spot method gave the same illumination. These were placed at determined distances from a glass jar containing leeches. While one bulb was kept constantly alight at a fixed distance, the other was moved foot by foot towards the leeches, thus providing for a range of light intensities. Leeches became responsive provided that the percentage reduction of illumination was at least 50 %. Below that figure there was no movement, but with increasing figures above 50 %, more and more were brought into a state of activity. The same percentage reduction of illumination was found necessary to effect the same response when both lights were near the leeches as when both lights were placed at a considerable distance.

If a piece of cardboard was held in the hand and moved so that the shadow cast passed over the cephalic half of a leech, the latter immediately became active. Such an effect was not seen when the caudal half was shaded. That the cephalic half should be receptive is to be expected from the position of the specialized sensillae, or eyes, which are present only on the 2nd, 3rd, 4th, 5th and 8th of the 34 annuli.

(*e*) *Attraction offered by skin*

Pieces of skin and their underlying connective tissues, which had been cut from dead rabbits, cats and rats, proved to be attractive to leeches, and continued to act as a diminishingly powerful attractant for several days. Leeches were unable to locate the bait at a distance, but once contact had been made, they stopped in their tracks, quickly prepared their incisions, and applied their mouth-parts to the wounds. The majority were content to suck for 40–60 min. without raising their heads, while one sucked for 110 min. and another for 120 min. Others did not settle down so well and withdrew their heads after 1–10 min. The latter, after one or two exploratory probing movements, would before long prepare a second incision nearby. If the inner or connective tissue side of the skin was placed uppermost, leeches accepted this surface with almost the same readiness. However, the greatest attraction of all seemed located in the cut edges, for more leeches settled down there than could be accounted for by chance selection. During their attempted feeding other stimuli failed to act. Neither human breath nor the rubbing of their bodies with a warmed glass rod caused them to remove their

heads. Whenever a leech left a proffered bait and was once again on the move, there was, however, a quick response to a current of warm moist air with a looping advance to reach the source, a display that is never seen after a true blood meal.

By contrast, pieces of rabbit muscle and rabbit fat which were placed in the path of leeches and were traversed by them provided but little interest, in that only about one leech in ten attempted to obtain blood.

Two pieces of rabbit skin were then selected, one piece being wrapped round a beaker containing warm water at a temperature of 40° C., and the other around a beaker containing cold water at a temperature of 18° C. Each proved to be attractive, although the former appeared to excite a slightly greater interest. When the same pieces of skin, with some loss of suppleness, were tested 3 days later, only about 50 % of the leeches would attempt to feed on the skin that was cold, but the skin wrapped around the beaker whose water had been rewarmed provided an interest as great as on the freshly removed skin.

Examination of the rabbit's skin revealed incisions which differed in no way from those inflicted on man when a true blood meal was obtained. It would seem that once the sucking reflex is established, leeches are oblivious to the fact that they are not filling their bodies with blood. Test-feeding or weighing before and after their attachment to the skin revealed that under surrounding conditions of 70 % relative humidity and a temperature of 21° C., they might lose as much as 30 % of their weight in 2 hr. On the other hand, when leeches are observed in the act of taking a true blood meal from the skin of man a clear fluid may be seen exuding from their bodies which, if undisturbed, increases in quantity and extends to form an almost complete protective covering. The fluid that is exuded throughout the period of the feed results from the concentration of blood. Under these latter conditions dehydration does not occur; the leeches become greatly distended and are found to have increased their weight many times.

That skin removed from dead animals and of the same temperature as the surroundings should be acceptable made it seem possible that the attraction might be due to some contained chemical constituent, a belief that was strengthened by the fact that if skin, and especially its cut edges, were pressed and rubbed on glass, the area so treated became covered with leeches. Their anterior suckers were applied so firmly that no amount of pulling of their bodies would make them let go. There was no indication that they were guided to the treated area from a distance.

In an attempt to identify this supposed chemical substance, pieces of shaved rabbit skin were ground up with sand and distilled water in a pestle and mortar, the resulting fluid being filtered and spread on pieces of glass. Other pieces of skin were treated with alcohol. When evaporation was complete the glass was offered to leeches by being placed in their paths, but on no occasion did either of the extracts provide any attraction. Leeches crossed the treated areas without pausing in their course.

As blood is the only known food, it might be assumed that this fluid, free and removed from its vessels, might establish the sucking phase, but this is not so, for, when leeches were induced to make contact with drops of freshly drawn blood, they passed their anterior suckers through the film and yet failed to pause and examine that which they had sought for so long.

IV. DISCUSSION AND CONCLUSIONS

Coagulation of the blood. Lindemann (1939) showed for the medicinal leech, *Hirudo medicinalis* Linn., that blood from the wound, after the leech falls off, coagulates in normal time and suggested that hirudin is never injected into the wound but is added to the blood as it passes through the pharynx, the entire bleeding phase being due to the action of histamine-like bodies preventing the natural closure of capillaries. The blood issuing from the wound made by the land-leech, however, initially has a long coagulation time, which diminishes until 8 min. after the removal of the leech. From then onwards the coagulation time is of normal duration. It would seem, therefore, that both hirudin and histamine-like bodies are injected into the wound, the two substances ensuring that an adequate supply of incoagulable blood is delivered into the pharynx. During the first few minutes following the removal of the leech from the skin, the blood issuing from the wound contains some hirudin-like substance which is soon washed out by the blood coming up from below. The continuation of the bleeding after these first few minutes is due solely to the action of the vasodilator substance, which, as in the medicinal leech, continues to act on the capillaries, inducing a prolonged paralysis in them. Accordingly, it was considered that thrombin, as a means of arresting the blood flow, could scarcely be expected to be of great assistance; moreover, an examination of the literature did not reveal any likely source of the large quantities which would be needed, nor did it seem practicable to make a preparation that could be relied upon to retain its activity under service conditions. Armed with these facts, doubt was felt as to the likelihood of shortening the bleeding time, and, indeed, no satisfactory method was found. Pressure alone will, in time, stop the flow of blood, while sealing with ordinary 'court plasters' has some effect. Certain protein precipitants, if closely applied, will shorten the time, especially in the case of bites

made by small leeches. Fused alum in the form of a pencil is probably the most useful of these precipitants; it is effective if the surface clot which results from its application is not disturbed. In practice, however, the clot which acts as a delicate seal is more often than not rubbed off and bleeding recurs. Adrenaline 1 in 1000 is ineffective, for the solution when applied to the skin never reaches the paralysed capillaries, since the opening is small and the flow is from below towards the surface.

A number of travellers have described ulcers and abscesses resulting from leech bites. No opinion can be offered as to whether these infections result directly from the bites or whether they are acquired through scratching and later contamination. In the course of experiments several hundred leeches were allowed to bite the arms and legs. Although a certain amount of irritation was experienced, care was taken not to scratch the skin. No antiseptics were used. When the flow of blood persisted for an unusually long time alum was sometimes applied. The wounds all healed quickly and without complications.

Repellents. The selected repellent should possess certain attributes. It should act promptly and should be chemically stable in the presence of the high humidity and temperatures of tropical climates. Its volatility should be low and it should not easily be washed off by soap, by rain, and sweat. Toxic effects to man, either local or general, should not occur. It should be capable of easy application, not greasy, and be free from unpleasant odour. Large quantities should be fairly readily available at reasonable cost. Finally, the same repellent should if possible be effective also against certain insects, in particular mosquitoes, which have always presented a far more serious problem than that under consideration.

Hydroxycitronellal was the most effective substance investigated. Unlike other citronella compounds it was effective over a long time. Its odour is slight but agreeable, and it has been shown to act as a repellent against mosquitoes. Supplies were limited, the price was high, and there was no certainty that a dermatitis might not be induced in certain individuals living under hot and humid conditions. For these reasons attention was turned to the four substances which stood next in repellent power, namely di-methyl phthalate, di-ethyl phthalate, ethyl hexanediol, and vanillin. Of these ethyl hexanediol was not available in quantity and, moreover, it was not found to be a successful repellent against the majority of disease-carrying mosquitoes. Di-ethyl phthalate and vanillin suffered from these disadvantages to an even greater degree. Only di-methyl phthalate fulfilled all the specified requirements, and it appeared to do so admirably. No hesitation was felt at the conclusion of the work undertaken in the Physiology and Pharmacology Department of St Bartholomew's Hospital in recommending its use in the field. It was felt that though a valuable measure of protection would be afforded by the application of the liquid to the skin and clothes where attacks were likely, a cream or paste would be more convenient to carry, for di-methyl phthalate is not easily confined to the usual types of liquid containers. Many preparations were made by Dr P. Hamill, M.D., D.Sc., F.R.C.P., Mr H. Davis, Ph.D., Chief Pharmacist to University College Hospital, and by Mr King, Pharmacist to Chase Farm, E.M.S. Hospital, Enfield. Of these the following were the most satisfactory:

(1)	Di-methyl phthalate	25
	White wax	18
	Arachis oil	57
(2)	Di-methyl phthalate	50
	White wax	10
	Arachis oil	10
	Wool alcohols	30
(3)	Di-methyl phthalate	50
	Wool alcohols	50
(4)	Di-methyl phthalate	25
	Lanette wax	25
	White wax	15
	Hard paraffin	15
	Wool fat	20

The preparation numbered (1) was the first choice, for it spreads well on skin and leather. Moreover, the wax and arachis oil augment the efficacy by delaying evaporation of the active material, thus retaining it longer in contact with the surface to which it is applied.

At a later date opportunities occurred for estimating the value of liquid di-methyl phthalate and several of these preparations in jungle-infested country. It was found that boots and shoes, if dry, imbibe di-methyl phthalate readily and remain repellent for several days, but that protection was best secured by special attention in treating the region of the eyelets and upper margins. Skin did not retain the repellent so well, reapplication being necessary after a period of 6–18 hr. A light covering of the liquid or cream to the footwear and to the skin of the leg as far as the knee sufficed to prevent attacks in country where without such aid bites would have been frequent. Under similar jungle conditions the value of hydroxycitronellal in affording protection was compared with that afforded by di-methyl phthalate. As both gave such good results it was not easy to make a decision, both being effective provided that no accessible surface was left uncovered. Nevertheless, the impression was gained, as in the laboratory tests, that hydroxycitronellal possessed the greater power to repel, and that it would be the substance of choice if the question of supply and cost were not

considered. Neither produced any irritant effect on the skin.

The effect of temperature. Land-leeches were not attracted to a warmed glass test-tube from a distance, but directly contact was made they showed interest and assumed the sucking position. They were able to follow both a current of warm moist air and the human breath, while their line of progress towards the source could be deflected by altering the direction of the current. Since atmospheric air and breath were equally attractive, it is unlikely that the latter contains a chemical attractant. Such behaviour leaves little doubt that temperature acts as a token stimulus.

The effect of illumination. The leeches displayed activity when they were subjected to a decrease of illumination, but with an increase of illumination they failed to change their position, a behaviour which has been observed in the past in other animals, and noted by Gee (1913) in the water-leech *Dina microstoma* Moore. In land-leeches the essential factor inducing a response was the percentage reduction in light intensity and not the magnitude of the reduction as measured in light units. This required percentage reduction in illumination was at least 50 %, but no animal is quite normal under laboratory conditions, and it may be that in the wild state a smaller reduction would suffice, and enable the near presence of man to be detected by the shadow that he casts.

Attraction offered by skin. That skin removed from animals and of the same temperature as the surroundings should, on contact, be attractive to leeches, made it seem likely that some contained chemical was acting as an attractant. An attempt was made to extract the substance but failed, either because it was not soluble in the two solvents used or else because it was presented in an unsuitable concentration.

V. SUMMARY

The descriptions which have been given by travellers leave no doubt that land-leeches can cause considerable annoyance, and that the protective garments and substances that were applied by them to the skin and clothing were unsatisfactory. It was thought that other and more elaborate clothing would not supply the answer, as they would be hot and uncomfortable to wear. Accordingly, a search was made for an efficient repellent. Altogether seventy-eight substances were examined, and of these hydroxycitronellal was the most powerful but expensive and not readily obtainable. Di-methyl phthalate was efficient and the most generally useful. As di-methyl phthalate is a liquid which easily escapes from containers, creams incorporating the substance were evolved. Formulae for these creams are given.

Evidence was obtained that two substances are injected into the tissues when the leech bites. One of these, a hirudin-like substance, is soon washed out of the wound by the blood coming up from below. Persistence of bleeding is due to a second substance which is similar in action to histamine and causes a capillary paralysis. No satisfactory method of shortening the bleeding time was found. Alum was of some help provided that the surface clot was not disturbed.

The behaviour of land-leeches and their response to certain physical and chemical stimuli were studied in the search for an efficient repellent.

Part of this work was undertaken under the guidance and close co-operation of Dr P. Hamill, whose advice had been sought as to what protective measures might be taken against land-leeches by those engaged in fighting the Japanese in Burma.

I desire to express my indebtedness to Sir Rickard Christophers for his kindness in placing at my disposal samples of substances which were being evaluated by him as possible mosquito repellents.

Sick Berth Petty Officer A. T. Page helped me to assess the coagulation times of blood. Both he and Sick Berth Chief Petty Officer H. W. Lee spent many days with me in leech country. I shall not easily forget their companionship and enthusiasm for a pursuit which was unusual and on occasion vexatious.

REFERENCES

BAILEY, F. M. (1945). *China-Tibet-Assam. A Journey, 1911.* London: Jonathan Cape.

BECCARI, O. (1904). *Wanderings in the Great Forests of Borneo.* London: A. Constable and Co. Ltd.

COULTER, J. L. S. (1933). *J.R. Nav. Med. Serv.* **19**, 105–8.

GEE, W. (1913). *Univ. Calif. Publ. Zool.* **2**, 197–305.

GREEN, P. E. (1907). *Spolia zeylan.* **4**, 191.

HAEKEL, E. (1883). *A Visit to Ceylon.* English Trans. Boston.

HAKLUYT, R. (1598). *Voyages*, **2**, 58.

HARDING, W. A. & MOORE, J. P. (1927). *The Fauna of British India, including Ceylon and Burma. Hirudinea.* London: Taylor and Francis.

HOOKER, J. D. (1854). *Himalayan Journals.* London: John Murray.

LINDEMANN, B. A. (1939). *Arch. exp. Path. Pharmak.* **193** (4).

MILLER, C. C. (1946). *Black Borneo.* London: Museum Press Ltd.

MOORE, J. P. (1932). *Rec. Indian Mus.* **34**, 1.

SHIPLEY, A. E. (1915). *The Minor Horrors of War.* London: Smith Elder and Co.

SMYTHE, F. S. (1930). *The Kangchenjunga Adventure.* London: Victor Gollancz Ltd.

TENNENT, J. E. (1859). *Ceylon: An Account of the Island, Physical, Historical and Topographical; With Notices of its Natural History, Antiquities and Productions*, 2 vols. London: Longman and Co.

WRIGHT, A. E. & COLEBROOK, L. (1921). *The Technique of the Teat and Capillary Glass-tube*, 2nd ed. p. 112. London: Constable.

(*MS. received for publication* 6. VII. 1948.—Ed.)

THE PENETRATION OF THE HOST TISSUE BY THE HARVEST MITE, *TROMBICULA AUTUMNALIS* SHAW

By B. M. JONES, *Zoology Department, University of Edinburgh*

(With 10 Figures in the Text)

CONTENTS

	PAGE
I. Introduction	247
II. Methods	248
III. Morphology of the organs related to the phenomenon of piercing and sucking	248
(1) Buccal apparatus	248
(*a*) The chelicerae	248
(*b*) Cheliceral muscles	249
(*c*) The maxillary palps and their muscles	251
(2) The digestive system	251
(*a*) Buccal cavity	251
(*b*) Pharynx and pharyngeal muscles	251
(*c*) The oesophagus and ventriculus	251
(*d*) The salivary glands	253
(3) The excretory organ	253
IV. Choice of habitat upon the host	254
V. The source of the food supply	254
VI. The mechanism of piercing	254
VII. The effects of the salivary secretion	255
VIII. The mechanism of suction	256
IX. Discussion	258
X. Summary	259
References	260

I. INTRODUCTION

Trombicula autumnalis Shaw belongs to the widely distributed group of Trombiculid mites. Our knowledge of the commonly occurring hexapod larval stage is very fragmentary, this being partly due to the group having presented an interesting field for taxonomic research and most attention has so far been directed to external morphological descriptions rather than to the physiological aspects of the biology of these mites. Moreover, the medical importance of the group became even more apparent during the war, since certain species were either suspected or proved to be the infective agents of a mite-borne typhus, an acute febrile disease, which occurred among the military forces in the Far East and this gave a new impetus to detailed investigations in the taxonomy and bionomics of the group.

The literature provides little information on the life cycle of these mites in their natural habitats, but breeding experiments in the laboratory have provided much valuable data. The larvae are found living freely upon the surface of the soil. They are potential parasites and given the opportunity they will attach themselves to a bird or mammal host. During the period of attachment, which normally lasts about 3–5 days for *T. autumnalis*, the larvae become fully engorged with nutrient material obtained from the superficial tissue of the host. The engorged larvae drop off the host on to the soil and metamorphose to the nymphal stage, which eventually changes to the adult stage to complete the life cycle. According to Keay (1937) and to my own observations, when carrying out breeding experiments with *T. autumnalis* it is essential for the larva to be fully fed. It must increase its size about three to four times before the change to the nymphal stage can take place.

Trombiculid mites do not at any stage in their life history introduce solids into their digestive tracts. The larvae are parasitic on animal hosts, whilst vegetable food is thought to meet the requirements of the nymphs and adults, although experiments have shown that they will partake of the contents of insects' eggs as well as the body tissue of small macerated arthropods (Jayewickreme & Niles, 1946, 1947). My own experiments also gave evidence that the nymphs of *T. autumnalis* will partake of the contents of *Forficula* eggs. The fact that Trombiculid mites as a group do not accept nourishment in any other form but that of a viscous liquid, is also observed in the Araneida and Insecta. The feeding process in these mites entails the ejection of the saliva which changes the nature of the host tissue by extra-intestinal digestion. During the feeding process a unique tubular tissue structure is gradually formed in the skin of the host and is connected temporarily to the buccal aperture of the mite.

In 1871 Gudden first observed the tubular structure in the skin of the host and since that time it has received the attention of Brandis (1879),

Trouessart (1897), Jourdain (1899) who gave to it the name 'stylostome', Toldt, jun. (1923) and André (1927) who preferred the descriptive term 'histio-siphon'. The many controversial interpretations and suggestions of previous workers regarding the structure and function of the 'stylostome' indicated that further investigation was required.

The literature affords little information on the morphology of the organs of *Trombicula autumnalis*, but the excellent descriptions given by Michael (1896), Henking (1882), and Sig. Thor (1904) for adult forms of other Prostigmata were very helpful. However, the structure of the larval forms, although simpler, differs in many ways from that of the adults, therefore before an attempt could be made to describe the physical mechanism associated with such an unusual feeding process, the anatomy of the larva of *T. autumnalis* had first to be investigated in some detail.

The means whereby the larvae obtained food from the host appeared to have been taken for granted. In order to understand this unique method of feeding a study has been made of the morphology and function of the organs of the larva which are related to the physical mechanisms of piercing and sucking. Detailed observations have also been made of the formation of the tissue canal and the cytological and physiological changes in the skin of the host brought about by the injection of an active secretion.

II. METHODS

Larval mites (*T. autumnalis*), living upon the surface of the soil, were obtained by means of a light trap method (Jones, 1947); and field mice (*Apodemus sylvaticus*), heavily infected with mites, were caught in the Colinton district of Edinburgh.

In order to observe the details of the cuticular structures larvae were mounted, straight from life or alcoholic Bouin's fixative, in polyvinyl alcohol (Jones, 1946). Host tissue, heavily infected with larvae in all stages of engorgement, was fixed in Bouin's fluid for not longer than 24 hr. before being transferred to 70% alcohol to await further treatment. Before embedding, the material was passed through amyl acetate. This is a convenient procedure since it eliminates the use of absolute alcohol and the material can be left indefinitely in the amyl acetate before being washed in toluol.

Individual specimens collected from the soil were similarly treated except that, after fixing, they were transferred to a glass tube 1 cm. in height and diameter and the serial changes were made by using a fine bore suction pipette. The end of the fine capillary was slightly oblique and when placed on the floor of the tube, allowed the intake of liquid whilst the mites remained undisturbed in the tube for the wax embedding.

Heidenhain's iron haematoxylin, Mayer's haemalum and alcoholic eosin were used as stains. The picric acid of the fixative was purposely not washed out of some preparations since it stained the cuticular structures of the larva so effectively.

III. MORPHOLOGY OF THE ORGANS RELATED TO THE PHENOMENON OF PIERCING AND SUCKING

(1) *Buccal apparatus*

The homology of the parts of the buccal apparatus of many Prostigmata has led to much discussion, but in *Trombicula autumnalis* the chelicerae and maxillary palps are recognized.

There is a central prolongation of the cephalothorax which forms a buccal cone or rostrum containing the pharynx and buccal aperture (Fig. 2, *p.*, *b.a.*). Upon the ventral surface of this cone are the fused coxal plates of the maxillary palps, whilst the posterior part of the dorsal surface extends as a deeply cleft plate which in dorsal view is lyre-shaped. (Figs. 2 and 4, *d.b.co.*)

(*a*) *The chelicerae*

The chelicerae (*c.*) are dorsal to the buccal cone and function as piercing organs. Each chelicera is composed of an apical claw or digit (*c.c.*) which articulates with a relatively large basal part. The chelicerae are more or less contiguous and their bases are covered by the anterior border of the thin cuticular region which precedes the dorsal scutum (Figs. 1–4).

The large basal portions may be described as boat-like structures. In dorsal view the upper surface of the basal portion is pointed anteriorly, broadening to a somewhat V-shaped posterior margin. The sub-apical region of the inner oblique border articulates with the basal portion of the claw, whilst the posterior region extends as a slightly curved spicule-like process which tapers to a point (Fig. 4, *c.p.*). A sagittal section through the basal portion of a chelicera shows the relationship of the chitinous margins to be similar to those of the upper surface (Fig. 3).

The distal part of the apical claw ends in a sharp point. The dorsal edge is concave with a sub-apical tooth whilst the convex ventral edge is indented just posterior to the position of the tooth. The lateral borders are relatively broad and flattened and curve proximally towards the base. Around the broadest part of the base is a fold or ridge which, upon the dorsal side, curves posteriorly to form a half-circle (Fig. 4, *b.f.*). In side view the base of the claw extends dorsally and the posterior margin is convex (Fig. 3, *c.b.*). The fold around the base appears to act as a flange for articulation with the sub-apical region

of the oblique border of the second larger part of the chelicera.

(*b*) *Cheliceral muscles*

There are two sets of cheliceral muscles within the cavity of the second basal part of the chelicera. The dorsal muscles arise from the upper inner region of the posterior surface and are inserted on the dorsal region of the basal portion of the claw (Fig. 3, *d.c.m.*). The ventral muscles are inserted on the ventral region of the base and are attached to the lower outer region of the posterior surface (Fig. 3, *v.c.m.*).

Since the claw articulates at an angle with the inner oblique surface of the second part of the chelicera, the posterior surface of the basal portion of the claw is obliquely placed so that the action of the dorsal and ventral muscles causes a rotary movement of the base. The dorsal set is composed of about ten muscles as opposed to only three ventral muscles, since upon their stronger action depends the rotation dorsalwards of the dorsal part of the base, whereby the claws are brought into position for piercing the skin, with their inner lateral borders and distal sharp points more or less contiguous. The actual thrust through the skin is brought about by the action of muscles attached to the posterior surface of the chelicerae. These are the oblique body muscles which are inserted by tendons on the dorsal scutum and

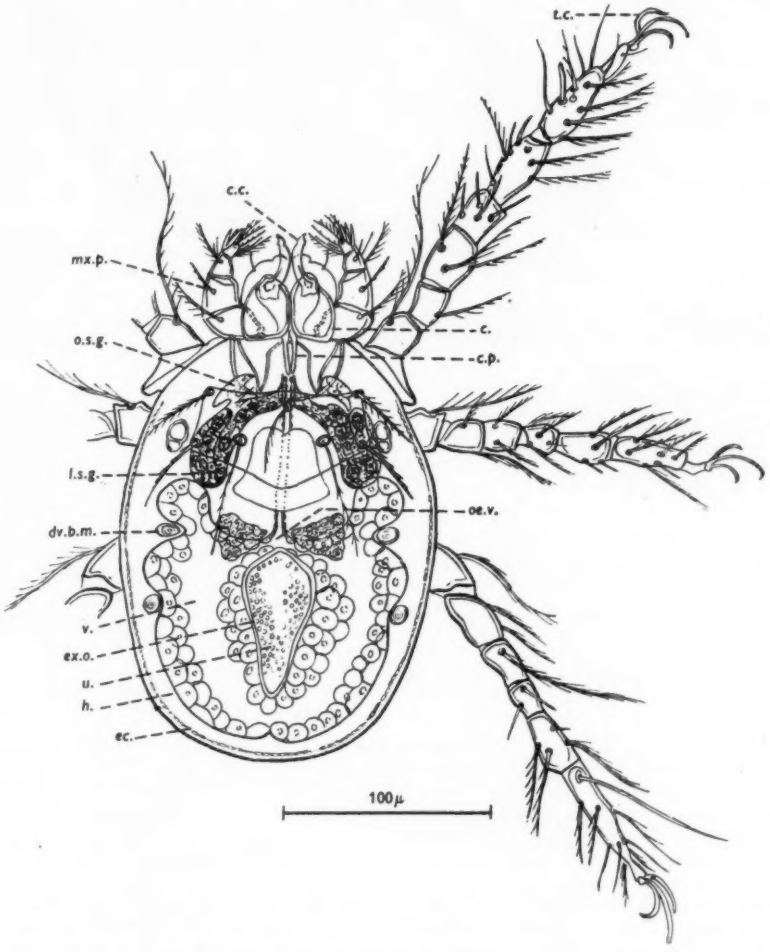


Fig. 1. Dorsal view of hexapod larva with a horizontal section superimposed to show the internal organs.

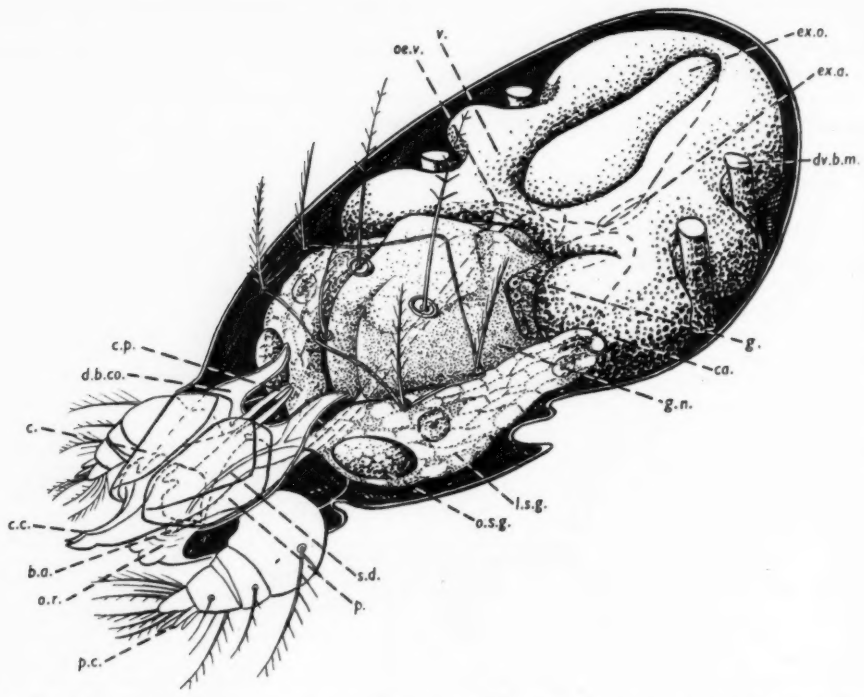


Fig. 2. Diagrammatic drawing of the larva *T. autumnalis* to show the organs associated with feeding.

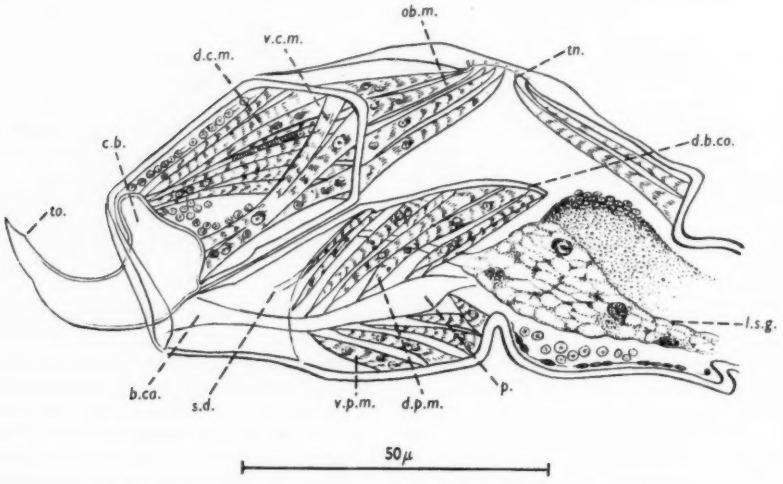


Fig. 3. Sagittal section to left of the mid-line of the buccal apparatus.

their action is responsible for the concerted forward movement of the chelicerae (Fig. 3, *ob.m.*).

(c) *The maxillary palps and their muscles*

A pair of short tactile palps is attached to the base of the buccal cone, and they curve towards their bases (Fig. 1, *mx.p.*). There are five articulating segments: the basal trochanter (Fig. 4, *tr.*); the femur, which is the larger segment (*f.*); the patella (*pa.*); and the penultimate tibia (*ti.*), which terminates in three claws, the middle one being the longest (*ti.c.*). The claws are opposed to the tactile papilliform tarsus (*ta.*) which represents the last segment. One of the functions of the maxillary palps is that of supporting the larva when it is attached to the skin of the host. In addition, the tibial claws and opposing tarsus are probably utilized for gripping.

The arrangement of the flexor muscles (Fig. 4, *fl.m.*) allows a rotary movement in the horizontal plane and the tarsi are capable of being brought into close proximity with the oral recess.

In horizontal section three muscles from the ventro-posterior surface of the buccal cone are seen to be inserted on the trochanter. Four muscles with thin wide bases arise from the posterior surface of the femur and are inserted by means of tendons on the distal margin of the anterior surface of the patella. Similarly, two muscles extend from the patella to the tibia and two muscles from the tibia to the tarsus.

In the distal region of the palp there is a ganglion with nerve cells and fibres running to the sensory hairs of the tactile tarsus. The amoeboid blood corpuscles, or leucocytes, which float and migrate in the plasma of the haemocoelic body cavities, are present in considerable numbers in the distal region of the palps.

(2) *The digestive system*

The digestive system of the larva of *Trombicula autumnalis* is composed of the oral recess, buccal cavity, pharynx, oesophagus, liver-stomach or ventriculus and an organ which appears to have an excretory function.

The liquid food enters the digestive tract through the pharynx and oesophagus into the ventriculus. The latter may be regarded as a blind sac whose folded walls are capable of distension. This is accompanied by a corresponding expansion of the thin corrugated cuticle of the epidermis, the mite thereby assuming the appearance of an opaque sub-oval bladder filled to capacity with food. The reddish pigment, so conspicuous in the free-living larva, is either changed or completely broken down during the feeding process. The loss of the red pigment at this stage is interesting, in view of the fact that the ectoparasitic period may be regarded as a phase preliminary to a hypogeal mode of existence, which is a characteristic feature of both the nymph and adult stages of *T. autumnalis*.

(a) *Buccal cavity*

The buccal cavity (Fig. 3, *b.ca.*) is distinguishable from the pharynx by the absence of attached muscles. The cuticle is thin and does not stain. The cavity widens anteriorly and opens into the oral recess (Fig. 2, *o.r.*).

(b) *Pharynx and pharyngeal muscles*

The cuticle of the pharynx is thin and unstained, but the cells of the attenuated ectodermal layer are deeply stained. The lumen is folded in cross-section and the pharyngeal muscles are arranged radially. By the action of these muscles the surfaces of the folds are rhythmically pressed together and drawn apart thus functioning as suction apparatus (Fig. 3, *d.p.m.*, *v.p.m.*).

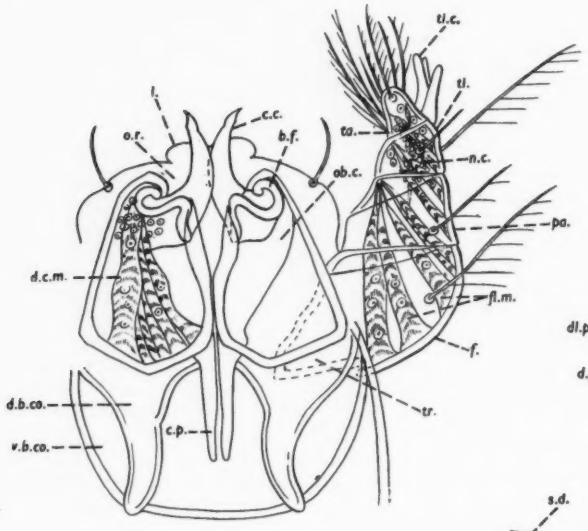
The muscles arise from the dorsal, ventral, and lateral surfaces of the buccal cone, and extend obliquely forwards to the pharyngeal wall to which they are connected by tendons. Two rows of muscles extend from the posterior region of the ventral surface of the buccal cone to the ventral side of the pharynx. Two rows of dorsal pharyngeal muscles are attached to the cleft dorsal surface, and there are two rows of dorso-lateral and two rows of ventro-lateral pharyngeal muscles (Figs. 5, 6 and 7, *dl.p.m.*, *vl.p.m.*). The rhythmical action of the longitudinally arranged system of muscles is responsible for the peristaltic movement of the pharynx, thus ensuring the continuous flow of liquid food.

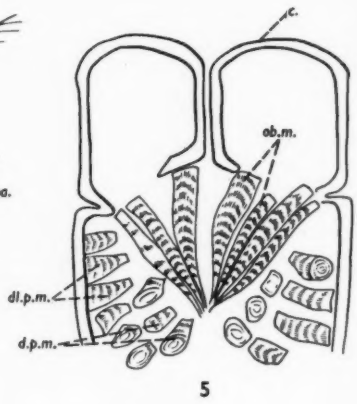
(c) *The oesophagus and ventriculus*

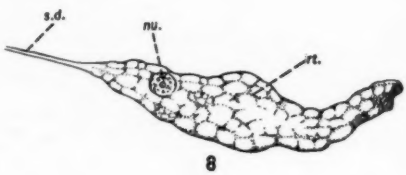
The oesophagus is slightly expanded where it arises from the hind end of the pharynx before it extends slightly upward and backward as a slender tube which runs through the ganglion complex. Almost immediately behind this, it enters by an oesophageal valve through the ventral surface of the anterior region of the central cavity of the ventriculus (Figs. 1, 2, *v.*). The oesophagus is somewhat shorter than the pharynx; the cuticle is thin and does not appear to take up any stain. The ectodermal layer, which is composed of darkly stained cells, is extremely attenuated, but a well-defined layer of ectodermal cells is formed around the cuticle of the funnel-shaped structure which projects into the cavity of the ventriculus as a sphincter-like valve (Figs. 1, 2, *oe.v.*).

The peristaltic movement of the pharynx ensures a continuous flow of liquid food along the oesophagus and through the oesophageal valve into the ventriculus which gradually distends as it receives the supplies of food. The oesophageal valve prevents the reflux of the liquid food into the oesophagus. The action of the dorso-ventral body muscles (Fig. 2,

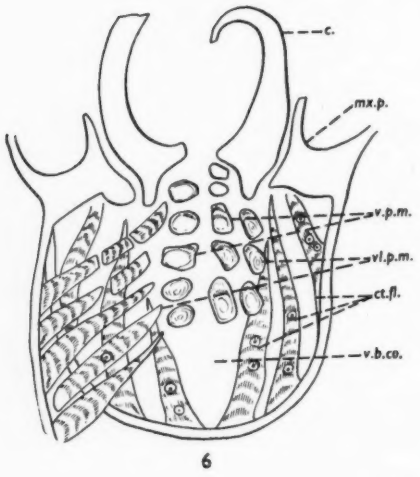
dv.b.m.), which run between the caeca of the ventriculus, is to aid the distribution of the food which flows into the central cavity, thereby eliminating any resistance to the continuous flow along the pharynx and oesophagus whilst the suction mechanism is in operation.







50 μ



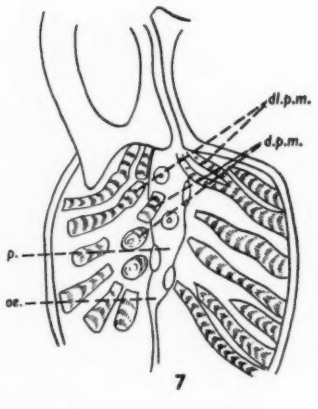


Fig. 4. Dorsal view of buccal apparatus; based on whole mounts and sections.
Fig. 5. Horizontal section through dorsal region of buccal cone.
Fig. 6. Slightly oblique horizontal section through ventral region of buccal cone.
Fig. 7. Slightly oblique horizontal section through dorsal region of buccal cone.
Fig. 8. Large salivary gland.

(d) *The salivary glands*

The group of glands in the anterior part of the body of many species of Prostigmatic mites, are referred to as the salivary glands. Sig. Thor (1904) discusses these glands and adopts Michael's retention of the term 'salivary glands' when describing them. He reviews previous work on the glands and gives the synonomy of the names previously used. He recognized six paired glands and one unpaired gland during his researches and found that they occurred in various combinations according to the species:

(1) Tubular salivary glands.
(2) Reniform glands.
(3) Dorsal anterior glands.
(4) Dorsal posterior glands.
(5) Oesophageal posterior glands.
(6) Oesophageal anterior glands.
(7) Tracheal gland.

It is difficult and sometimes impossible clearly to homologize these glands. Suggesting homologues for the glands found in the larval mite presents an even more difficult task. Henking (1882) describes two pairs of glands for the larva of *Trombidium fuliginosum* Herm. The larger (*schlingen formige*), looped, glands and the small oval glands, closely applied to the anterior surfaces of the former as shown in his diagram.

Two pairs of salivary glands are found in the larva of *Trombicula autumnalis* but, whereas the relationship of the glands with one another is similar to that found in *Trombidium fuliginosum*, the shape, structure and position of the larger glands differ from those described and figured by Henking.

The posterior surface of the small oval gland is closely pressed against the front end of the large gland when the two are *in situ*, so that in section it is difficult to distinguish them (Fig. 1, *o.s.g.*, *l.s.g.*). These small glands have an affinity for haematoxylin, but their structure is not well defined. They are probably homologous with the oval glands of *T. fuliginosum*.

The relatively large pair of salivary glands lie one on each side of the oesophagus and extend outward and backward around the ganglion complex with their apices in close proximity with the front end of the antero-lateral caeca of the ventriculus. In horizontal section (Fig. 1, *l.s.g.*) the glands appear to increase in width towards rounded posterior margins. In lateral view, the gland which is approximately 80 μ in length, tapers somewhat towards both ends, with its greatest width of 18 μ across the middle position (Figs. 3, 8). The duct, composed of a very thin non-staining cuticle, seems to arise from the anterior part of the gland and extends forward and upward over the pharynx between the pharyngeal muscles to just below the roof of the buccal cone before extending downward to the buccal cavity region (Fig. 3, *s.d.*).

The large salivary glands are composed of large cells provided with large spheroid nuclei and nucleoli. The cytoplasm is granular and stains deeply with haematoxylin and presents more the appearance of a deeply stained reticulum with scattered nuclei rather than well-defined cells containing distinct nuclei. The nucleus is often indistinct and sometimes cannot be distinguished. The staining of the gland as a whole is sometimes uneven, the distal part being more granular and composed of a very darkly stained irregular reticulum. The histological appearance of the gland depends upon whether the mite examined was collected whilst free-living or in the act of feeding, and this indicates that it is correlated with secretory activity. The glands of larvae collected from the surface of the soil during their pre-parasite phase of existence are more granular, the irregular reticulum of the cytoplasm stains very darkly, and the nuclei are not easily distinguished. In contrast, the glands, during the ectoparasitic feeding phase frequently appear to be less granular and the cytoplasmic reticulum and nuclei stain lightly.

These large glands are relatively larger than those described by Henking for the larva of *T. fuliginosum*. The histological appearance of the cells with their granules, large spheroid nuclei and deeply staining cytoplasmic reticulum, resembles that of the pericibal salivary glands of *Bdella* described by Michael. He also suggested that the pericibal glands with their ducts opening into the epipharynx, were true salivary glands.

It would appear, therefore, that the large salivary glands of the larva of *Trombicula autumnalis* are not homologous with the looped glands of the larva of *Trombidium fuliginosum* and that their histological appearance closely resembles that of the pericibal salivary glands of *Bdella*.

The salivary glands with their large polygonal cells and axial duct have been likened to malpighian tubules. Owing to the mites feeding on pre-digested nutriment the malpighian tubules are thought to have lost their original excretory function during the evolution of the Prostigmata, eventually becoming cut off from the gut to take on their new role and opening by a common duct near the front end of the mite.

The saliva is ejected by action of the body muscles contracting the haemocoel, the pressure thus exerted upon the glands causing them to exude their secretory contents which are forced through the narrow lumen of the salivary duct.

(3) *The excretory organ*

In dorsal view the malpighian-like excretory organ is more or less pear-shaped, lying along the

median line of the ventriculus and dividing the latter for most of its length (Figs. 1, 2, *ex.o.*). In section the walls of the organ lie very close to the inner walls of the ventriculus. The epithelium of the wall of the excretory organ, consists of cells which are not well defined.

The organ has not been clearly homologized. Extra-intestinal digestion has probably been responsible for the apparent loss of continuity between the lumen of the hind gut, and that of the ventriculus. The hind gut, no longer required for rejecting indigestible material, gradually evolved as an excretory organ. Sections of larvae collected from the soil clearly show the presence of urates in the lumen of this organ (Fig. 1, *u.*). No opening between the excretory organ and the ventriculus was detected and, although one or more may exist, the liquid food in sections of engorged larvae would not extend them sufficiently to facilitate observation. The absence of an opening would not affect the excretory function, since, except for the dorsal surface, the walls of the organ are closely applied to those of the ventriculus, thereby permitting the passage of urates from the ventriculus, through its thin outer membrane, into the lumen of the organ.

IV. CHOICE OF HABITAT UPON THE HOST

The choice of habitat varies according to the host. Larvae prefer to attach themselves to regions of the body which provide the most favourable physical conditions. The amount of hair and the exudation of secretions influence the choice and the inside of the ear and the area around the anus of mammals are characteristic habitats. Relatively few observations have been made on birds but the mites appear to favour the anal area and the lateral surface of the body between the wing and the thigh but are more concentrated around the ptagium and the crural fold (Gill, Moule & Rick, 1945).

The factors which influence the choice on man are more varied, partly owing to the different kinds of apparel worn, but the regions of the body where the skin is thin and which provide the necessary warmth and humidity favoured by the larvae are most likely to be attacked. The thinness of the horny epithelium is an important factor, since the actual piercing is accomplished by the minute cheliceral claws. The humidity preference shown by the larvae during their free-living existence and the fact that warmth increases their activity (Jones, 1947) makes it understandable that mites leaving the humid conditions of the soil will crawl over the surface of the body until those sites which provide a moist, warm, habitat are reached. Typical sites are the waistline, axillae, genitals, groin and ankle. There is, of course, no limit to the sites which may be attacked and an extremely susceptible host will show the skin reaction on less typical parts of the body, whereas immune individuals will not be affected at all.

V. THE SOURCE OF THE FOOD SUPPLY

Prevention of infection is the function of the horny covering of the skin. A mechanical or chemical irritant which penetrates the horny covering will provoke changes in the underlying malpighian layer which, by the process of keratinization, forms resistant tissue. The malpighian layer is soft and spongy and the intercellular spaces are filled with tissue fluid, the distinct intermediate product between blood plasma and lymph. The composition of tissue fluid corresponds closely to that of blood plasma, except that the protein content is almost negligible (Wright, 1945). The pre-digested nutrient material which is taken into the digestive tract of the larva consists of the tissue fluid and the disintegrated malpighian cells which have come into contact with the injected salivary secretion.

Examination of prepared sections of the skin of the hosts selected for study showed that penetration by the saliva never extended beyond the malpighian layer.

VI. THE MECHANISM OF PIERCING

The initial piercing of the skin is done by the minute cheliceral claws, the movement and fixation of which are controlled by the cheliceral muscles. The bases of the claws are capable of a rotary movement. When free-living forms are examined from the dorsal aspect, the claws are seen to lie either with their broad lateral borders in the horizontal plane and the convex or concave edges facing inwards, or with the convex edge of one facing the concave border of the other depending on the action of the attached muscles. In readiness for the act of piercing the bases rotate so that anteriorly their inner lateral borders are more or less contiguous, with the pointed apices directed dorsalwards. The claws are rigidly held in this position by the contraction of both sets of cheliceral muscles when they puncture the skin.

Observations were made of the behaviour of the larva during the act of piercing human skin, but a more intimate view was obtained by observing, under the microscope, larvae piercing a membrane which had been stretched over the open end of a tube containing serum. In this preliminary attempt at artificial feeding, a fresh piece of mouse gut was stretched over the open end of a glass tube 0·5 cm. in diameter. When dry it adhered firmly to the glass. A cork was placed in the other open end of the tube which was then placed in a glass cell containing serum. The serum diffused through the membrane and when the tube contained sufficient serum to maintain a film under the membrane, the tube was

removed. Larvae were placed upon the membrane, and their behaviour suggested considerable excitement compared to their relative activity in a dry tube.

The activity of the larva during the act of piercing the membrane, which was held horizontally under the microscope, was performed by vigorous movements. The palps appeared to grip the surface and gave support, whilst the action of the legs caused the body to tilt upwards from the horizontal to an angle of roughly 45°, and a general burrowing action ensued before the larva lowered its body and finally became quiescent. The behaviour suggests that the forward tilting movement of the body facilitates the entry of the sharply pointed apices of the fixed hook-like claws accompanied by the forward movement of the chelicerae, whilst the lowering action of the body follows as the claws make their curving penetration under the surface. The efficiency of this hook-like attachment is appreciated when an attempt is made to remove a larva in the act of feeding. As the body becomes swollen with food, it rests at an angle to the surface of the skin, supported by the palps, whilst the legs, now relatively undersized, are directed posteriorly at the sides of the body.

In dorsal view the claws present the appearance of a spear-head, since the space between them widens towards their bases. Therefore the opening of the puncture increases in width as the claws are buried in the skin. Observations of prepared sections of tissue (Fig. 10B), show the claws, *in situ*, slightly parted anteriorly. This suggests that, after entry, they rotate slightly to enlarge the puncture and facilitate the subsequent passage of the saliva through the horny epithelium. This layer is notoriously resistant to ferments, therefore the presence of a thick horny epithelium may be important in preventing the injection of the saliva into the underlying malpighian cells. The claws can only penetrate to a distance of 20–25 μ, and for this reason the sites on the body of the host where the horny layer is thin, are more favourable.

The irritant effect produced by this physical process provokes changes in the malpighian layer. Sections of host tissue showed that the claws, *in situ*, prior to any injection of saliva had caused localized keratinization (Fig. 10A). This indicated that the malpighian cells react quickly to the puncture and that the injection of the saliva does not necessarily immediately follow the piercing action of the chelicerae.

Further penetration into the malpighian layer is achieved by the hydrolysing action of the saliva which is injected under pressure. The oral recess of the larva is applied to the orifice of the puncture and the gap is sealed by the product resulting from the action of the saliva upon the surrounding epithelium. The contraction of the body presumably exerts a pressure upon the large salivary glands and the saliva is forced through the salivary duct and injected into the space between the embedded claws. The propulsion force and dissolving action of the saliva are responsible for the complete penetration through the horny layer. Thereafter, the conflicting actions of the saliva and the malpighian layer take place.

VII. THE EFFECTS OF THE SALIVARY SECRETION

When the larva is securely attached to its host, the feeding process begins and lasts usually 3 or 5 days. During this period the intermittent injections of saliva are responsible for the formation of a tissue canal.

The saliva is forced through the tissue fluid of the intercellular spaces of the malpighian layer. The saliva comes into contact with the cells and causes a marked disintegration of the cell contents. The cytoplasm becomes disorganized and the nuclei are reduced to irregular-shaped fragments which stain more deeply. The nutrient material which enters the digestive tract of the mite is composed of the liquefied tissue and the granular break-down products (Fig. 9, *d.cyt.*, *d.nu.*, *n.m.*).

The irritant effect of the saliva also causes an increased effusion of tissue fluid which plays a part in the complex phenomenon—'inflammation'—which forms a characteristic feature of the skin reaction in man to the irritating physical and chemical processes instigated by the ectoparasitic larva.

The tissue canal is a tube with a definite opaque wall, around which a mass of hyaline tissue is deposited in layers (Fig. 9, *t.c.w.*, *p.ti.*). There is good evidence that the hyaline tissue represents keratinized malpighian cells and that the separation of the layers by modified malpighian cells is responsible for the ringed appearance of the structure. The hyaline mass is soluble in a solution of sodium sulphide in caustic soda and before dissolving it swells, thus indicating the presence of keratin. Moreover, the mass has an affinity for Heidenhain's iron haematoxylin stain and the modified layers of malpighian cells stain blue-black, which is characteristic of malpighian cells undergoing keratinization. The wall of the canal, in some sections, also showed a marked affinity for the stain. Therefore I do not regard the previous use of the word 'homogeneous' (Toldt, jun., 1923) as a correct description of the hyaline mass and do not agree that it is formed entirely of solidified secretion (Jourdain, 1899; and André, 1927), although a substance analogous to that produced by the silk glands of the closely related Tetranychids might be present.

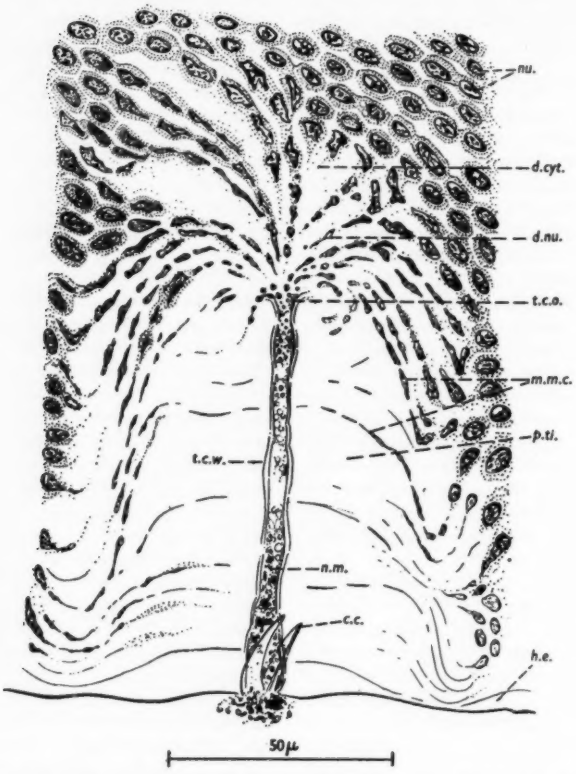


Fig. 9. Median longitudinal section through tissue canal and surrounding protective tissue showing streaming of disorganized host tissue towards distal opening of canal during the act of suction.

VIII. THE MECHANISM OF SUCTION

It has already been mentioned that, owing to the action of the saliva, the buccal opening of the mite is sealed to the orifice of the puncture in the skin of the host. Examination of sections and the fact that, on detaching the larva carefully, both the suction canal and the hyaline tissue adhere to the oral recess of the mite, clearly show that there is a physical continuity between the proximal end of the tissue canal and the buccal cavity. This is an important factor in considering either of the two conflicting functions—that of suction or that of ejection.

When the claws have been forced into the desired part of the skin of the host, the saliva is ejected. At first the saliva injected into the malpighian layer is forced into an area which may be described as mushroom-shaped, with the 'stalk' tapering towards the buccal aperture of the mite (Fig. 10C). The malpighian cells, which come into contact with the saliva, disintegrate and become partially liquefied. If the oral recess is sealed to the orifice of the puncture the sucking action follows. No structure resembling a tissue canal is observed at this stage, therefore the peristaltic movement of the pharynx causes the direct entry of the nutrient material into the digestive tract (Fig. 10C).

As the pharyngeal muscles contract, they pull out the walls of the pharynx, thus greatly enlarging the lumen of that organ and the suctorial effect is produced by a reduction of pressure within the pharynx. The peristaltic action of the pharyngeal muscles ensures a flow of the distintegrated cells and tissue fluid into the buccal cavity, along the pharynx and oesophagus, and through the oesophageal valve into the ventriculus. The increased effusion of tissue fluid towards the source of irritation aids the flow of nutrient material.

As the activating saliva is sucked back into the digestive tract, the malpighian cells, following in the wake of the liquefied disorganized tissue, lay down protective tissue. The flow then decreases, the

sucking action stops, and another injection of saliva is given. A passage through the newly deposited protective tissue is made and the saliva spreads like a fountain and affects the malpighian cells within a relatively wide arc (Fig. 10F). The flow of disorganized liquefied tissue is again produced and the sucking action recommences.

The alternation of the two contradictory processes is controlled by the time which the host tissue takes to overcome the activity of each injection of saliva which is largely taken back into the digestive tract with the nutrient material.

The intermittent ejections of saliva are responsible for the form of the structures which gradually take shape in the host tissue. As the liquefied host tissue and activating saliva are sucked away, the

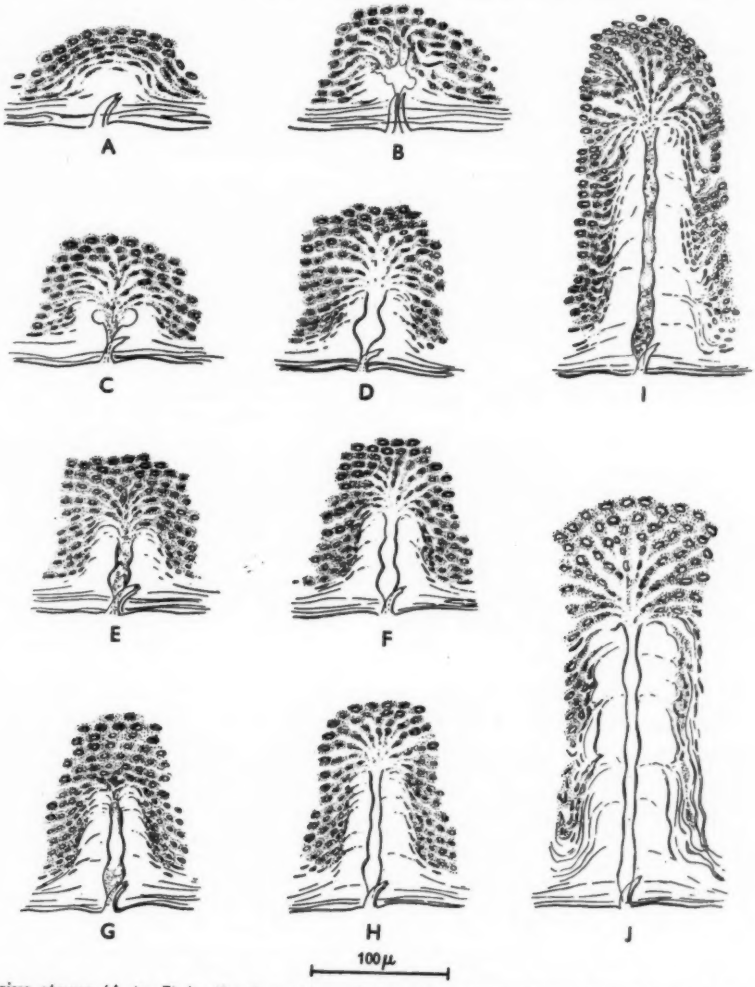


Fig. 10. Successive stages (A to J) in the formation of the tissue canal shown in relation to the alternating processes of ejection of saliva and that of suction of nutrient material. A. Reaction of malpighian layer to the physical irritation produced by the piercing claws. B. Initial injection of saliva. C. Initial suction of disorganized host tissue. D. Tissue canal forming. E. Suction and deposition of protective tissue. F. Injection of saliva through newly deposited protective tissue. G. Suction arrested by the deposited protective tissue and lack of active saliva. H. Injection of saliva through protective tissue and extension of tissue canal. I. Suction and flow of nutrient material along well-formed tissue canal. J. Increased keratinization around well-formed canal.

malpighian cells lay down a new zone of protective tissue, and the passage is lined with a deposit which forms the insoluble wall of the tissue canal (Fig. 10G). During the period of feeding, the alternation of the ejecting and sucking processes is responsible for the deposition of the superimposed layers of protective tissue, and the gradual growth in length of the tissue canal.

The influence of the intermittent injections of saliva is also shown in the structure of the tissue canal by the successive dilatations of the wall. The conspicuous dilatations at the proximal end correspond to the initial injections, but thereafter the walls straighten and the dilatations are not so clearly discernible. The distal opening is usually more or less funnel-shaped (Fig. 9, *t.c.o.*).

The formation of the tissue canal introduces another factor, that of capillarity. The extent to which capillarity assists in the suction process is difficult to determine, since it depends upon (*a*) the surface tension and density of the liquefied tissue, and (*b*) the diameter of the canal. Of these points, only the diameter of the tissue canal can be determined (see Fig. 9), and in the case of such a fine bore as that of the canal, the capillarity should be appreciable unless the nutrient material is more viscous than it appears.

The formation of the tissue canal changes the sequence of events in the suction process, for with the reduction of pressure or tension in the pharynx a pull will first be exerted upon the fluid in the canal. The effusion of tissue fluid towards the source of irritation, and the capillarity, if operative, will combine with the peristaltic action of the pharyngeal muscles to ensure a continuous flow of nutrient material along the tissue canal, pharynx and oesophagus, for the period during which the preceding injection of saliva remains effective.

The factor of capillarity must also be considered as a resistance to the propulsion force of the ejected saliva. Examination of sections of the host tissue with well-formed tissue canals *in situ*, shows that the resistance of capillarity, if it does exist, presents no difficulty to the ejection of the saliva through the fine bore of the canal. Moreover, there is no decrease in the radius of the arc of host tissue which is affected by the saliva when it is forced out from the distal end of the relatively long canal (Fig. 10J).

The number of factors which play a part in controlling the length, bore, or form of the tissue canal are so numerous that measurements are not of much value, but a well-formed tissue canal can attain a length greater than that of the larva.

IX. DISCUSSION

Awati (1914), in describing the mechanism of suction in *Lygus pabulinus* Linn., the potato capsid bug, stated 'that an important thing for suction is that suction and ejection canals must be separate and distinct from each other. The least intercommunication will stop the whole mechanism. Also one canal cannot serve two contradictory purposes—one of sucking and the other of ejecting saliva. The whole mechanism therefore depends upon the presence of two canals.' In the light of this statement, which is true of piercing and sucking insects (Grove, 1919), it is interesting that the Trombiculid larva when it parasitizes a host, efficiently uses one canal, viz. the tissue canal, as a duct for both the ejection of saliva and the suction of food.

The relationship between the unique tissue canal and the physical mechanisms of ejection of saliva and that of suction, whereby the larvae obtain food from the host, appears either to have been taken for granted or not to have been elucidated because the structure and function of the 'stylostome' or 'histiosiphon' in the skin of the host, remained obscure.

Previous workers have given their attention to the presence of a tubular structure in the skin of the host. In 1871, Gudden observed, in the course of a diagnosis of thrombosis in man, that from this ectoparasite (*Trombicula autumnalis*) protruded a tubular organ penetrating the skin and he considered the tube to be a permanent organ and supposed that the apparatus was retracted inside the body whilst the mite was free-living. Brandis (1879) also maintained that the tubular organ possessed an external cuticle which continued internally to line the internal canal.

Jourdain (1899) considered that the tubular organ was a temporary structure and gave to it the name 'stylostome'. Trouessart (1897) stated that the central canal contained a tongue process, which functioned in the same way as a piston in the body of a pump and he claimed to have seen this tongue process *in situ*. Brandis later admitted the temporary nature of the stylostome and thought that the tissue of the host formed part of its structure. Toldt, jun. (1923) described the stylostome as composed of a homogeneous, transparent, colourless or hyaline material with a clear and vitreous central canal.

In 1927, André reviewed the suggestions of previous workers as to the structure and function of the stylostome and emphasized a statement by Jourdain that the walls were formed from solidified secretion which was probably produced by the salivary glands. It also appeared to André as if, at its periphery the tubular organ had produced a modification of the host tissue and, therefore, he assumed that a main function of the walls of the tube was to react with the host tissue and transform it into nutritive material suitable for ingestion by the mite.

In the present paper, as the result of observations, chemical tests and staining methods, a different

interpretation is given for the structure of the tubular mass, to that given by previous workers, who called it 'stylostome' or 'histiosiphon'.

It is suggested that the axial canal of the 'stylostome' or 'histiosiphon' is the tissue canal and the surrounding hyaline mass is not formed of solidified secretion but is made principally of superimposed layers of keratinized tissue deposited as a protection against the chemical and physical irritants.

The tissue canal may be compared with the thin tubular sheath which is formed around the mouth-parts of plant-feeding aphids (Davidson, 1923), but, whereas the insoluble wall of the tubular sheath in plant tissue is thought to be composed of calcium pectate, the wall of the tissue canal is probably formed of insoluble protein.

André (1927), after careful search, stated that he had not detected a structure comparable with the tongue process which Trouessart claimed to have seen in the axial canal of the 'stylostome'. My own observations of both the tissue canal and the buccal apparatus of the larva confirm the statement that no such process appears to be present.

Gudden and André observed that the axial canal of the 'stylostome' was sometimes filled with nutrient material, whilst Toldt referred to the canal as being clear and vitreous. The dual function of the tissue canal accounts for the different observations. During the suction process, liquefied tissue and granular material are present in the canal but during the alternate ejection process, the presence of the salivary secretion only gives the canal a clear vitreous appearance (Fig. 10A–J).

The interpretation of the tubular structure in the skin of the host as a tube with a definite wall surrounded by keratinized protective tissue, indicates that the feeding mechanism involves a single duct, the tissue canal, and the physical processes of ejection of saliva and suction of food. The previous suggestions that the surrounding tissue, or wall of the 'stylostome', was formed of a solidified secretion tended to obscure the functions of the tubular mass. The hydrolysing action of the saliva is of prime importance when injected into the skin of the host and this property is just as essential in the digestive tract of the mite since the nutrient tissue entering the buccal cavity still contains some granular material. It is natural, therefore, that the saliva should be sucked back into the gut with the food. The loss of saliva to form a mass around the axial canal, as suggested by previous workers, would be uneconomical.

The unique principle of the tissue canal functioning as a single duct for the two alternating purposes — ejection of saliva and suction of food—therefore served as a basis for understanding the relationship between the intermittent injections of saliva, the formation of the tissue canal and the deposition of protective tissue.

It is interesting that the larvae of some Hydrachnids, which are closely related to the Trombiculids, are parasitic on mosquitoes and also produce similar tubular structures in the tissue of the host (Marshall & Staley, 1929). However, whereas the tubular structures of Trombiculid mites penetrate in a more or less straight line at a variable angle to the surface of the skin of the host, those produced by the Hydrachnid larvae may be curved or looped, although they appear to lie near the surface.

X. SUMMARY

1. The introduction refers to the fragmentary state of our knowledge on Trombiculid mites and indicates that most attention has been devoted to the taxonomy and bionomics of the group. The life cycle and general method of feeding are briefly outlined and reference is made to previous work directly concerned with the present treatise.

2. The morphology of the organs related to the phenomenon of piercing and sucking in the larva of *Trombicula autumnalis* is described.

3. The physical factors of the skin influence the choice of habitat upon the host.

4. The food consists of tissue fluid and disintegrated cells of the malpighian layer partially liquefied by the action of injected saliva, a condition typical of extra-intestinal digestion.

5. The mechanism of the initial piercing of the skin, which is done by the cheliceral claws, is described.

6. A tubular tissue canal is gradually formed in the skin of the host and around its insoluble wall are deposited superimposed layers of keratinized protective tissue.

7. The mechanism of suction is described and the factors which play a part are considered. The problem of the tissue canal serving as a duct for both the ejection of saliva and the suction of food is explained by the alternation of the two processes. The inter-relationship, between the intermittent injections of saliva, the formation of the tissue canal and the deposition of protective tissue, is elucidated.

REFERENCES

André, M. (1927). *Bull. Mus. Hist. nat., Paris*, **38**, 509–16.

Awati, P. R. (1914). *Proc. Zool. Soc. Lond.* **2**, 685–733.

Brandis, F. (1879). Festschr. 50en Bestehen Provinz. Irrensanst. Nietleben.

Davidson, J. (1923). *Ann. Appl. Biol.* **10**, 35–54.

Gill, D. A., Moule, G. R. & Rick, R. F. (1945). *Aust. vet. J.* **21**, 22–31.

Grove, A. J. (1919). *Parasitology*, **11**, 456–88.

Gudden (1871). *Virchows Arch.* **52** (5F. 2Bd.).

Henking, H. (1882). *Z. wiss. Zool.* **37**, 553–663.

Jayewickreme, S. H. & Niles, W. J. (1946). *Nature, Lond.*, **157**, 878.

Jayewickreme, S. H. & Niles, W. J. (1947). *Nature, Lond.*, **160**, 578.

Jones, B. M. (1946). *Proc. R. Ent. Soc. Lond.* A, **21**, 85–6.

Jones, B. M. (1950). *Parasitology*, **40**, 1–13.

Jourdain, S. (1899). *Arch. Parasit. Z.* no. 1.

Keay, G. (1937). *J. Anim. Ecol.* **6**, 23–5.

Marshall, J. F. & Staley, J. (1929). *Parasitology*, **21**, 158–60.

Michael, A. D. (1896). *Trans. Linn. Soc. Lond. Zool.* **6** (ser. 2), 477–528.

Thor, Sig. (1904). *Ann. Sci. Nat.* **19** (ser. 8, Zoology), 1–190.

Toldt, K. (Jun.) (1923). *Wien. klin. Wschr.* no. 33.

Trouessart, E. L. (1897). *Bull. Soc. ent. Fr.* no. 4.

Wright, S. (1945). *Applied physiology*, 8th ed. London: Oxford University Press.

Key to lettering of the text-figures

b.a.	buccal aperture
b.ca.	buccal cavity
b.f.	basal fold
c.	chelicera
ca.	caecum of ventriculus
c.b.	base of cheliceral claw
c.c.	cheliceral claw or digit
c.p.	cheliceral process
ct.fl.	coxo-trochanter flexor muscles
d.b.co.	dorsal wall of buccal cone
d.cyt.	disorganized cytoplasm
d.c.m.	dorsal cheliceral muscles
d.nu.	disintegrated nuclei
d.p.m.	dorsal pharyngeal muscles
dl.p.m.	dorso-lateral pharyngeal muscles
dv.b.m.	dorso-ventral body muscles
ec.	ectodermal layer
ex.a.	excretory aperture
ex.o.	excretory organ
f.	femur
fl.m.	flexor muscles
g.n.	ganglion complex
g.	proximal part of tubular gland
h.	haemocoel
h.e.	horny epithelium
l.	lower lip or labium
l.s.g	large salivary gland
m.m.c.	modified malpighian cells
mx.p.	maxillary palp
n.c.	nerve complex
n.m.	nutrient material
nu.	nucleus
ob.c.	oblique surface of chelicera
ob.m.	oblique body muscles
oe.	oesophagus
oe.v.	oesophageal valve
o.r.	oral recess
o.s.g.	oval salivary gland
p.	pharynx
pa.	patella
p.c.	palpal claw
p.ti.	protective tissue
rt.	reticulum
s.d.	salivary duct
ta.	tarsus
ti.	tibia
ti.c.	tibial claw
tn.	tendon
to.	tooth
tr.	trochanter
t.c.	trifurcate claw
t.c.o.	distal opening of tissue canal
t.c.w.	wall of tissue canal
u.	urates
v.	ventriculus or 'liver-stomach'
v.b.co.	ventral wall of buccal cone
v.c.m.	ventral cheliceral muscles
v.p.m.	ventral pharyngeal muscles
vl.p.m.	ventro-lateral pharyngeal muscles

(*MS. received for publication* 7. VIII. 1948.—Ed.)

MORTALITY OF *LAEMOPHLOEUS* (COLEOPTERA, CUCUJIDAE) INFECTED WITH *MATTESIA DISPORA* NAVILLE (PROTOZOA, SCHIZOGREGARINARIA)

By L. H. FINLAYSON, Ph.D.

From the Department of Zoology, University of Birmingham

(With 4 Figures in the Text)

It was noticed that some stock cultures of *Laemophloeus minutus* Olivier and *L. ferrugineus* Stephen were showing an abnormally high mortality. The numbers of living larvae appeared to decrease, many pupae died inside their cocoons and most of the few adults which emerged died within a few weeks. As adult *Laemophloeus* live for about 6 months at the conditions under which the cultures were kept (25° C. and 70 % R.H.) it was apparent that something was killing the beetles at all stages in development. Microscopic examination of dead larvae and adults which had been chopped up and mounted in Berlese's fluid (Swan's formula) revealed huge numbers of the lemon-shaped spores (sporocysts) or pseudonavicellae of a sporozoan parasite. Living larvae from infected cultures were dissected in saline, and smears were fixed in Bouin's fluid (aqueous) and stained with Heidenhain's haematoxylin. Other larvae were fixed in Bouin's fluid, sectioned, stained with Ehrlich's or Heidenhain's haematoxylin and eosin, cleared in xylol and mounted in Canada balsam. These preparations revealed various stages of a schizogregarine which appeared to be *Mattesia dispora* which was described by Naville (1930) from *Ephestia kühniella* Zell. (Lepidoptera, Phycitidae) and recorded by Musgrave & Mackinnon (1938) from *Plodia interpunctella* Hbn. (Lepidoptera, Phycitidae). Careful examination of sections and measurement of various stages confirmed this identification. All stages were found, with the possible exception of young gamonts formed by macronuclear schizogony.

As in *Ephestia* and *Plodia* the parasite lives in the fat body. Sporocysts, and probably sporozoites also, occur in the blood. Several sectioned larvae showed enormous numbers of sporocysts obliterating the fat body and filling the entire body cavity. Also present were numerous sickle-shaped bodies which were almost certainly sporozoites which had hatched out from some of the sporocysts. No other stages were present, which renders it improbable that these bodies were merozoites. Furthermore, they were smaller than the merozoites described by Naville (see Table 1 for dimensions). The emergence of sporozoites in the same host was described by Naville in *Ephestia* and suspected by Musgrave & Mackinnon in *Plodia*.

Table 1. *Dimensions of various stages of* Mattesia dispora

Stage	Mean size (μ)	Range (μ)	No. of individuals	Preparation
Sporozoites	$11{\cdot}3 \times 1{\cdot}5$	$9–15 \times 1–1{\cdot}8$	10	Section of larva
Sporocysts (spores)	$11{\cdot}8 \times 5{\cdot}7$	$11{\cdot}4–12{\cdot}3 \times 4{\cdot}2–6{\cdot}6$	5	Section of larva
Sporocysts (spores)	$12{\cdot}8 \times 6{\cdot}9$	$11{\cdot}9–13{\cdot}1 \times 6{\cdot}2–7{\cdot}6$	5	Berlese's fluid
Sporocysts (spores)	$12{\cdot}1 \times 7{\cdot}4$	$11{\cdot}4–13{\cdot}1 \times 7{\cdot}2–7{\cdot}6$	5	Saline*
Totals	$12{\cdot}2 \times 6{\cdot}7$	$11{\cdot}4–13{\cdot}1 \times 4{\cdot}2–7{\cdot}6$	15	Saline
Gamonts	6·6 (diameter)	—	2	Section of larva
Gametocysts	8·0 (diameter)	6·6–9·5 (diameter)	10	Section of larva

* Formula for saline (Baker, 1945, p. 182): NaCl, 0·7 g.; H_2O, 100·0 ml.; 10 % solution of anhyd. $CaCl_2$, 0·2 ml.

The dimensions of the sporocysts are less than those given by Naville ($14 \times 7{\cdot}5\,\mu$). Musgrave & Mackinnon also found that the sporocysts in *Plodia* were smaller than those described by Naville; the maximum size found by them was $13{\cdot}5 \times 8{\cdot}5\,\mu$. The other stages measured by Musgrave & Mackinnon agreed in size with those described by Naville. In the present case the measurements of the other stages agree with those given by the previous authors

except for the gametocysts which are smaller than those described by Naville which were 8–12 μ in diameter.

In order to investigate quantitatively the effect of *Mattesia* on a population of *Laemophloeus* an experiment was set up using *L. minutus* as host. Three sets of 2 × 1 in. tubes were set up in three desiccators containing KOH solutions controlling the relative humidity at 70 %. The desiccators were kept at a constant temperature of 25° C. In each desiccator there were twelve tubes, four containing twenty females each, four containing twenty males each and four containing twenty larvae each (1–9 days old). All the adults were from the same stock culture and were not more than a few weeks old. The food placed in each set of tubes (about 1·8 g. per tube) was different; set no. 1 was a control containing whole wheat flour treated to kill mites by heating at approximately 58° C. for 24 hr. (Food treated in this way will be referred to as 'sterilized'.) The other two sets contained food from two infected cultures (whole wheat flour + 10 % dried brewer's yeast) plus an equal amount of sterilized whole wheat flour. The infected food had been passed through a 40-mesh per inch sieve to remove all the dead adults and most of the larger larvae. It had then been treated to kill mites by heating at approximately 58° C. for 24 hr. ('sterilized') or by drying at 50 % R.H. at room temperature for 4 days. This dried food was equilibrated at 70 % R.H. before use. The three sets of tubes at the beginning of the experiment contained therefore the following:

Set 1. Sterilized whole wheat flour (control).

2. Sterilized infected food + sterilized whole wheat flour.

3. Dried infected food + sterilized whole wheat flour.

The living adults in each tube were counted every few days. The results are shown in graphical form in Figs. 1–3. Fig. 1 gives the numbers of adults found in the tubes started with twenty females. In the control tubes there was a normal increase in population but on the infected food there was a gradual decrease. In the case of the sterilized infected food there were emergences of F_1 adults between 60 and 80 days which were offset by deaths among the parent females so that there was no increase in total population. On the dried infected food no F_1 adults were obtained; all offspring died in the larval stages. Similar results for the tubes started as twenty larvae are shown in Fig. 2. Counting was deferred until the adults were due to emerge (about 50 days from oviposition at 25° C. and 70 % R.H.). Again the mortality was higher on the dried infected food than on the sterilized. The graphs show the numbers of live adults only, but on the dried infected food one dead adult was found showing that altogether twenty-five of the eighty larvae developed into adults. On the sterilized food three dead adults were found showing that seventy-seven out of the eighty larvae developed into adults. In neither case were any F_1 adults produced. The males (Fig. 3) gave very different results from the females and the larvae. Even after the populations in the female and larvae tubes had almost completely died out, the males had lost only a small proportion of their numbers. They showed a slightly more rapid fall in numbers on the sterilized infected food than on the dried or control.

The difference in mortality rate between populations started as females or larvae and purely male populations is probably due to a combination of two factors: (*a*) the larger number of insects present in the former owing to reproduction (after a longer period in the case of populations started as larvae); and (*b*) the eating of dead larvae by other larvae and adults. Where larger numbers are present the infection can spread more rapidly by disintegration of dead insects. The adults appear to be too heavily sclerotized to be eaten; larvae must be present for cannibalism to be important. Dead larvae are often found with large chunks eaten out of them. Where adults alone are present spores can be disseminated only by disintegration of the dead insects. Males probably eat much less than females but it is difficult to believe that this has any significance in lessening infection of males over so long a period as 128 days, especially in view of the fact that schizogony and hatching of spores occurs in one host. As the life cycle of the parasite is short (see below) only a few initial spores should be required to produce a lethal infection within a few weeks. It would appear, therefore, that adult males are more resistant to attack by *Mattesia* than females and larvae but this is by no means certain because cultures which are infected are often wiped out completely, males and females alike being killed off. Dead males have been examined and frequently found to be full of spores.

Fig. 4 shows the result of a preliminary experiment at 25° C. and 70 % R.H. which was carried out at the beginning of this investigation before the causative agent had been identified. The adults of *L. minutus* used in this experiment were unfortunately not sexed. They were all the same age. Curve *A*, on Fig. 4, shows the mortality rate in two tubes of 20 adults on infected food (flour + 10 % dried brewer's yeast). Curve *B* is the same for two other similar tubes and curve *C* is the mean for these four plus one other. It will be seen that the mortality rate was constant in all tubes. Curve *D* gives the mortality rate in two control tubes containing whole wheat flour. Deaths occurred within 20 days on the infected food and by 40 days 25 % were dead. In the controls the first deaths occurred around 50 days. These were probably due to infection also because at that time it was not realized how easily the infection could be transmitted and slightly less care was taken to avoid

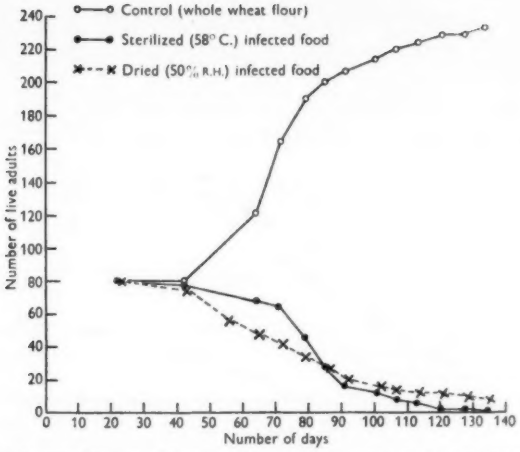


Fig. 1. Live adult populations of *Laemophloeus minutus* at 25° C. and 70% R.H. started as eighty females on infected and uninfected foods.

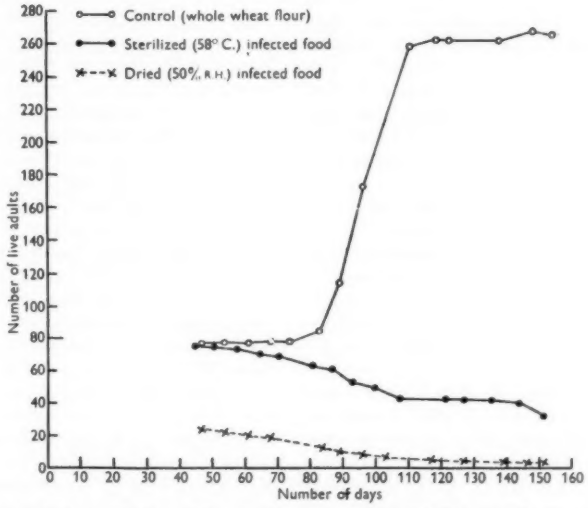


Fig. 2. Live adult population of *Laemophloeus minutus* at 25° C. and 70 % R.H. started as eighty larvae (1–9 days old) on infected and non-infected food.

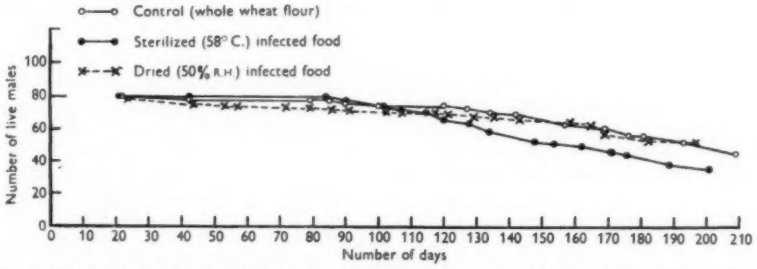


Fig. 3. Live population adult male *Laemophloeus minutus* at 25° C. and 70 % R.H. started as eighty males on infected and non-infected food.

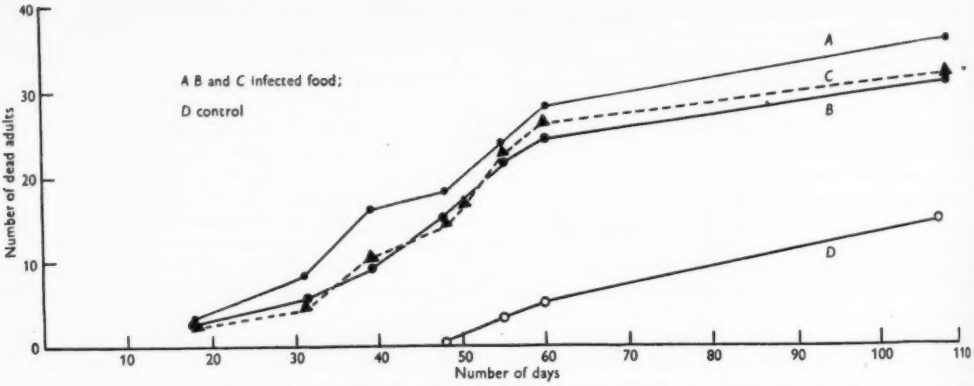


Fig. 4. Mortality rates of adult *Laemophloeus minutus* on infected and non-infected foods at 25° C. and 70% R.H.

contamination of the controls than in the later experiments. Although the infected food medium had previously supported a culture population, only about 100 F_1 adults had emerged instead of at least 1000 as obtained in normal cultures of the same size. There was, therefore, no possibility of food shortage being responsible for the more rapid mortality rate on the infected medium.

It is possible that mites act as carriers of spores, but no intermediate host or vector is necessary for the transmission of the parasite. Naville suggested that Ichneumons or Braconids might act as vectors but Musgrave & Mackinnon did not find any Hymenoptera in their cultures and there were certainly none in any of the *Laemophloeus* cultures used in the present investigation.

Four species of *Laemophloeus* have been exposed to infection by placing them on food containing spores obtained by grinding up dead adults and larvae. *L. minutus*, *L. ferrugineus* and an unidentified species (Lucas & Oxley, 1946) are readily infected and killed, but *L. turcicus* Grouve. appears to be immune. Many cultures of *L. turcicus* have been examined but no cases of infection have been found.

As a further check on the identification of the parasite, larvae of *Ephestia kühniella* and *Plodia interpunctella* were fed with infected flour. Both were attacked and killed by the sporozoan.

Recently Ghélélovitch (1948) has described a new genus of schizogregarine, *Coelogregarina ephestiae*, which also lives in the fat body of *Ephestia kühniella* and is also highly pathogenic. It is a monosporic form unlike *Mattesia* which is disporic. Both *Mattesia* and *Coelogregarina* were discovered because of their pathogenic nature; but it is possible that they also live in other insects in which they are non-pathogenic or less so, thereby resembling other schizogregarines and the cephaline gregarines which are so common in the gut of insects. The effect of cephaline gregarines on their hosts has not been investigated properly, but in view of their large size and often large numbers it must be considerable in some cases. Cephaline gregarines (*Gregarina laemophloei*—Foerster, 1938) were present in varying numbers in most of the *Laemophleous* examined; and in some of the *Ephestia* larvae very large cephaline gregarines (*Leydiana ephestiae*?—Ghélélovitch, 1948) were present in great numbers, making the gut contents appear white and granular to the naked eye.

REFERENCES

Baker, J. R. (1945). *Cytological Technique*. London: Methuen.

Foerster, H. (1938). *Z. Parasitenk.* **10**, 157–206.

Ghélélovitch, S. (1948). *Arch. Zool. exp. gén.* **85**, 155–68.

Lucas, C. E. & Oxley, T. A. (1946). *Ann. Appl. Biol.* **33**, 289–93.

Musgrave, A. J. & Mackinnon, D. L. (1938). *Proc. R. Ent. Soc. Lond.* (A), **13**, 89–90.

Naville, A. (1930). *Z. Zellforsch.* **11**, 375–96.

(*MS. received for publication* 1. IX. 1948.—Ed.)

THE PLEROCERCOID LARVA OF *GRILLOTIA HEPTANCHI* (VAULLEGEARD)

By GWENDOLEN REES, D.Sc.
Department of Zoology, University College of Wales, Aberystwyth

(With 18 Figures in the Text)

The plerocercoid larvae of *Grillotia heptanchi* (Vaullegeard) used in the present study were obtained from the right supra-orbital lateral line canal of a specimen of *Merluccius merluccius* (L.). Two larvae were located in the canal in the region of the frontal bone and two in the region of the nasal. All were alive and were found to expand and contract slowly on removal. Unfortunately, the exact locality from which the fish came is not known as it was obtained from a fishmonger at Cardiff, but it is fairly certain that it was caught off the west coast of the British Isles.

The adult worm has been recorded on several occasions from the Mediterranean. It was found at Nice by Meischner (1840) and Wagener (1854) in *Hexanchus griseus* (Bonnaterre), and at Naples by Oerley (1885) in *Heptanchus cinereus* (Gmelin) and *Torpedo ocellata* Rud., by Zschokke (1887) in *Hexanchus griseus* (Bonnaterre), *Heptanchus cinereus* (Gmelin) and *Scymnorhinus licha* (Bonnaterre), and by Joyeux & Baer (1936) in *Hexanchus griseus* (Bonnaterre) and *Scymnorhinus licha* (Bonnaterre). Other records of its occurrence are by Wardle (1933) from *Heptanchus cinereus* (Gmelin) in the Straits of Georgia, by Shen Tseng (1933) from *Notorhynchus platycephalus* (Tenore) at Tsingtao and by Hart (1936) from *Hexanchus griseus* (Gmelin) in the Puget Sound.

The larval stage was first found by Meischner (1840) in the intestinal wall of *Hexanchus griseus* (Gmelin) at Nice and later by Wagener (1854) in the same host species from the same locality. It was recorded by Pintner (1896) from the stomach of *H. griseus* (Gmelin) at Naples and by Joyeux & Baer (1936) from a specimen of *Lepidopus argentatus* Gouan taken from the stomach of *Heptanchus cinereus* (Gmelin) also at Naples. Two other records of it are by Wardle (1933) from the muscles in the vertebral region of *Theragra* spp. in the Straits of Georgia and by Hart (1936) from the muscles of *Ophiodon elongatus* (Girard) in the Puget Sound.

Neither the adult nor the larva has previously been recorded from British waters. Of the host species which harbour the adult worm, two, namely, *Hexanchus griseus* (Gmelin) and *Scymnorhinus licha* (Bonnaterre), occur in British waters, but the fishes from which the larva has so far been recovered, with the exception of *Hexanchus griseus* (Gmelin), do not occur in the waters around these islands. The larva has not so far been recorded from *Merluccius merluccius* (L.) nor from the unusual position of the lateral line canal.

Descriptions of the larval form are very brief and there has been some confusion over its identification. Wardle (1933) identified it as the larva of *Grillotia erinacea* (van Ben.), Hart (1936) as the larva of *Tentacularia* sp. and Joyeux & Baer (1936) as the larva of *Grillotia institata* (Pintner). Dollfus (1942) has given a list of the synonyms of both adult and larva. It was thought that the following more detailed description of the larva might facilitate future identification.

The plerocercoid larva is pear-shaped, the broader end being anterior (Fig. 1). It measures 9–10 mm. in length and 2·42 mm. in breadth at its widest part, behind which it narrows gradually to a diameter of 1·26 mm. near the posterior extremity. The scolex is contained in a cavity at the broader end, the cavity measuring 2·31 mm. in length and 1·63 mm. in maximum breadth, it opens to the exterior anteriorly by a circular pore 0·043 mm. in diameter (Figs. 1, 2). In *in toto* preparations the course of the excretory vessels and lateral nerve cords posterior to the scolex are easily seen. A dorsal and a ventral vessel pass down each lateral margin to open posteriorly into a small excretory bladder which communicates with the exterior by the excretory pore. The excretory vessels and lateral nerve cords emerge from the scolex and enter the body of the larva near the bases of the proboscis bulbs (Fig. 2). The excretory and nervous systems will be referred to in more detail later.

The scolex (Fig. 3) can be dissected out from the cavity in which it lies. When straightened it measures 6·63 mm. in length and is divisible into pars bothridialis, pars vaginalis and pars bulbosa. The maximum diameter of 0·90 mm. is attained in the pars bulbosa, the pars vaginalis being somewhat narrower and having a diameter of 0·63 mm. The depth of the scolex, dorso-ventrally, between the bothridia is 0·50 mm. There are two bothridia, one dorsal and one ventral, each measuring 1·20 mm. in length and 0·90 mm. in breadth. They are oval in

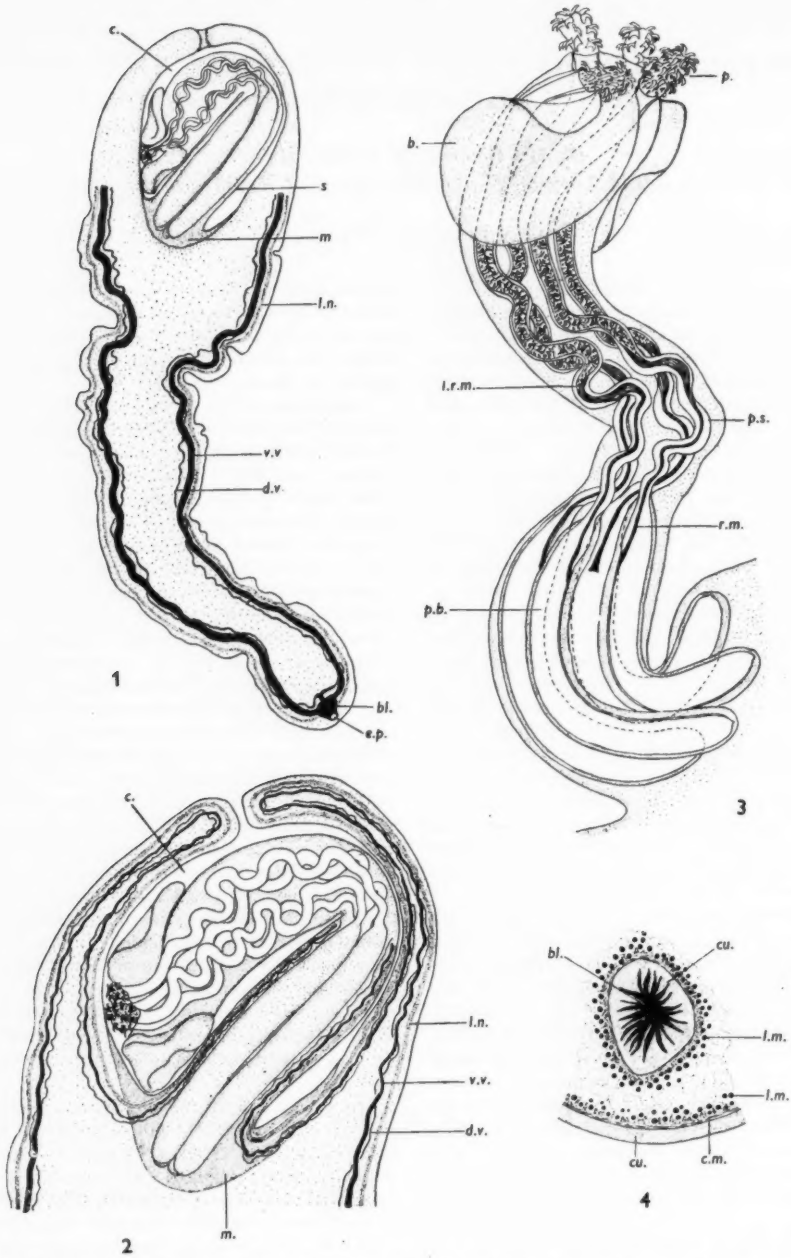


Fig. 1. Plerocercoid larva showing scolex in anterior cavity and lateral excretory vessels and nerve cords in body of larva.

Fig. 2. Anterior extremity of larva.

Fig. 3. Scolex.

Fig. 4. Transverse section through excretory bladder.

outline and inclined slightly forwards so that they converge anteriorly and diverge posteriorly. The scolex is provided with four armed protrusible proboscides opening immediately in front of the bothridia. In the larval stage the proboscides are almost entirely invaginated, only a small portion being protruded (Fig. 5). This, however, is sufficient to show the arrangement of the proboscis hooks. The arrangement of the hooks on the proboscis of the adult has been described by Dollfus (1942); the arrangement in the larval stage is similar (Figs. 6, 7). On each side of the proboscis is a slightly obliquely placed semicircular row of six hooks, the rows alternate with one another and are repeated at regular and fairly close intervals. Hooks 1–5 (1′–5′) follow one another closely, while hook 6 (6′) is some little distance behind hook 5 (5′). Hooks 1 and 1′ are the stoutest hooks of the series and are on the internal face of the proboscis (Fig. 6), the area between them being devoid of spines. Hooks 6 and 6′ are on the external face of the proboscis (Fig. 7) and differ slightly in shape and size from the others. Between hooks 6 and 6′ is a longitudinal band of smaller hooks or spines, and between the successive rows of large hooks on either side there are three or four rows of up to six spines each. The spines at the extreme tip of the proboscis are very small and more or less uniform in shape and size. It is probable that this portion is never evaginated (Fig. 5). The diameter of the proboscis, excluding the hooks, is 0·15 mm.

The proboscis sheaths pursue a sinuous course through the pars vaginalis but are not spirally coiled (Fig. 3). They measure 0·08 mm. in diameter. The wall of the sheath is cuticular and is lined by a layer of cells. The proboscis bulbs are relatively long measuring 2·20 mm. in length and 0·32 mm. in breadth; they lie parallel to one another in the pars bulbosa. The wall of each bulb consists of three layers, an outer cuticular layer continuous with the wall of the sheath, an inner layer of flattened cells lining the cavity and continuous with the lining layer of the sheath, and an intermediate layer of muscles comprising the bulk of the wall (Figs. 8, 9). The muscle layer consists of a number of lamellae arranged in two oblique series crossing one another and is not of uniform diameter. The wall on the outer side decreases rapidly in thickness to the point where the muscle fibres have their origins and insertions; the lumen of the bulb is therefore towards the outside (Figs. 5, 8 and 9). A band of cells which are probably myoblasts accompanies each bulb, lying along the median side throughout its length (Figs. 5, 8). They are closely connected with the muscles of the wall, the cuticular covering of the bulb being absent along the line of contact (Fig. 8). The lumen of the bulb is filled with fluid.

The retractor muscle of the proboscis originates from the wall of the bulb about one-fifth of the distance along its length and on the inner side (Figs. 3, 5 and 8). Some of the fibres of the retractor continue close to the inner wall of the bulb throughout its length (Fig. 5). From the bulb the retractor muscle passes forwards into the lumen of the proboscis sheath (Figs. 5, 9) and is inserted on to the apex of the proboscis (Figs. 5, 10). The muscle fibres encircle the apex of the proboscis, forming a thicker layer on the inner side and are inserted on to the proboscis wall in successive layers.

MUSCULATURE

Muscles of body. The superficial musculature of the body consists of an outer layer of circular and an inner layer of longitudinal fibres lying immediately below the basement membrane (Figs. 4, 11–14). At the posterior extremity the longitudinal muscles turn forwards around the excretory pore and continue as a band around the excretory bladder (Fig. 4), being inserted on to the cuticular lining at the anterior end of the bladder.

The parenchymatous musculature is poorly developed consisting of scattered longitudinal, transverse, dorso-ventral and radial fibres (Fig. 11). This arrangement persists throughout the body of the larva as far forwards as the base of the scolex. At the base of the pars bulbosa where the bulbs extend for a short distance into the anterior end of the body of the larva there is an aggregation of muscle fibres arranged in a circular manner around the bases of the four bulbs so that they become demarcated from the remainder of the parenchyma (Figs. 1, 2, 12 and 13). These circular muscles do not form a very definite band but spread out towards the periphery, some becoming continuous with scattered transverse fibres (Figs. 12, 13). In the invaginated state these circular muscles extend forwards for some little distance into the part of the larval body which envelops the scolex (Figs. 2, 13), where they gradually become more diffuse (Fig. 14) and finally disappear. In the evaginated state this means that there is a gradual concentration of circular fibres at the anterior end of the body of the larva where the pars bulbosa becomes continuous with it.

Muscles of scolex. The muscles of the scolex comprise those of the scolex itself, those associated with the proboscides and those of the bothridia. The superficial musculature of the scolex is the same as and continuous with that of the body. The scattered deeper longitudinal fibres present in the body become more concentrated dorsally and ventrally when they enter the scolex; they extend forwards through the pars vaginalis and enter the dorsal and ventral bothridia. The majority enter at the posterior end of the bothridia; but some continue forwards, entering

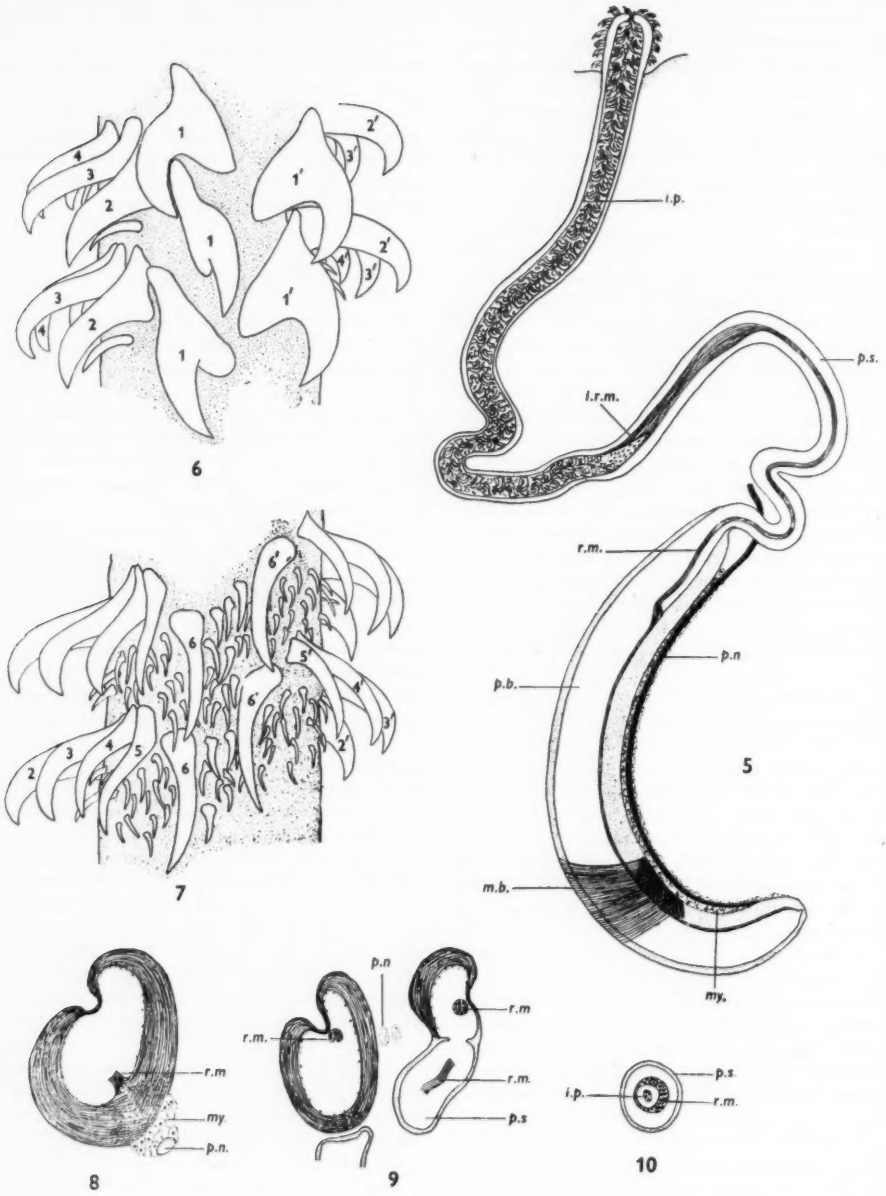


Fig. 5. Single proboscis.
Fig. 6. Armature of internal surface of proboscis.
Fig. 7. Armature of external surface of proboscis.
Fig. 8. Transverse section through proboscis bulb.
Fig. 9. Transverse section showing transition from proboscis bulb to proboscis sheath.
Fig. 10. Transverse section through proboscis sheath showing insertion of retractor muscle on apex of proboscis.

at various levels forming an ill-defined internal limiting layer to the bothridia. Within the bothridia these fibres become continuous with the delicate radial muscles which extend throughout these structures. The remaining muscles of the scolex are modified in connexion with the proboscides. Throughout the pars vaginalis and pars bothridialis is a well-developed band of transverse muscle fibres dorsally and ventrally external to the proboscis sheaths (Fig. 15). These presumably help to retain the proboscis sheaths in position during invagination and evagination of the proboscis itself. The radial muscles are better developed in the scolex than in the body, the fibres being inserted on to the outer sides of the proboscis sheaths.

There are well-developed muscles associated with the actual openings of the proboscides. Most conspicuous of these are a dorsal and a ventral transverse band of anterior muscles extending between the anterior extremities of the two dorsal and the two ventral proboscis sheaths immediately behind the apertures (Fig. 15). Immediately behind these are oblique muscles passing from the right dorsal to the left ventral and from the left dorsal to the right

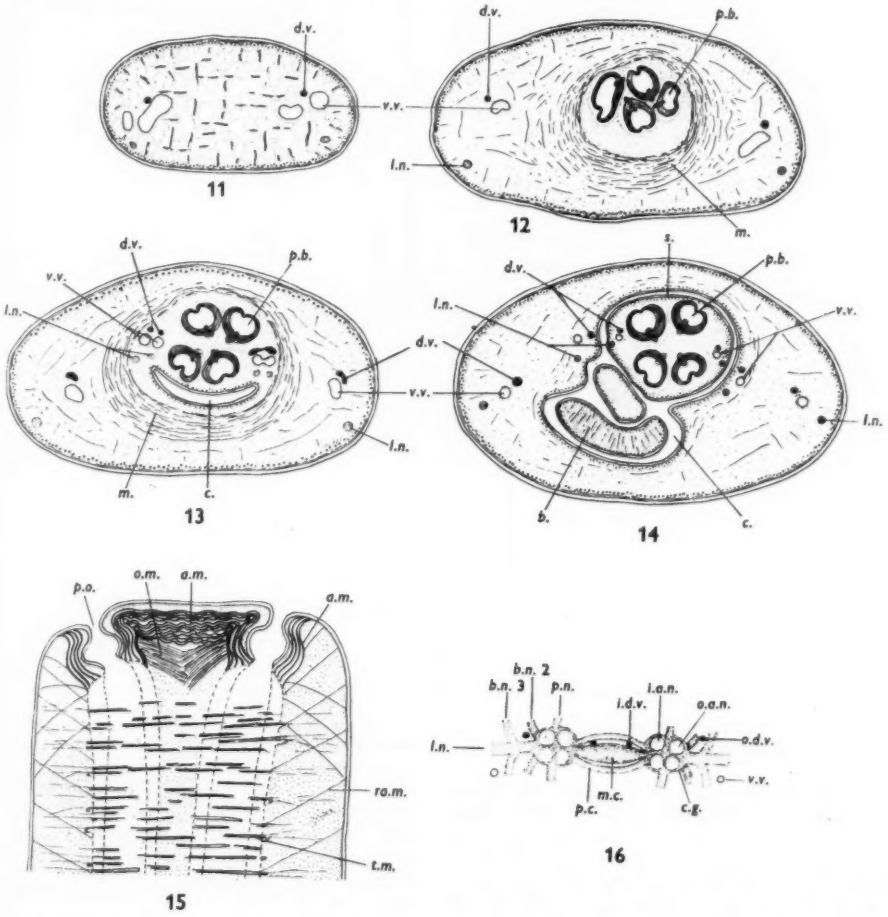
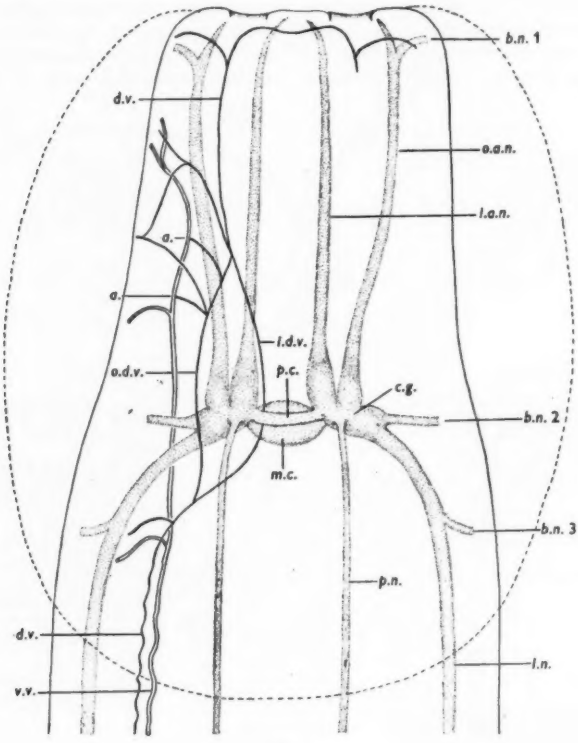


Fig. 11. Transverse section through body of larva near posterior end showing poorly developed musculature.
Fig. 12. Transverse section showing circular muscles around bases of proboscis bulbs.
Fig. 13. Transverse section near point of emergence from scolex of excretory vessels and lateral nerve cords.
Fig. 14. Transverse section showing scolex in cavity and arrangement of excretory vessels and nerves in this region.
Fig. 15. Longitudinal (horizontal) section showing arrangement of muscles at anterior end of scolex.
Fig. 16. Plan of brain, associated nerves and excretory vessels, anterior view.

ventral sheaths. On the outer side of each proboscis sheath at the same level is a separate bundle of anterior muscles inserted on to the cuticle just external to the proboscis pore. These three sets of muscle fibres presumably control the opening and closing of the proboscis pores and so facilitate their protrusion or withdrawal.

with one another by transverse commissures which pass parallel to one another across the middle of the scolex. There is a dorsal and a ventral commissure of fairly narrow uniform diameter throughout (Figs. 16, 17). Between the dorsal and ventral commissures is a third or median commissure connecting the right and left ganglionic masses (Figs. 16, 17). The median



17

Fig. 17. Dorsal half of nervous system, excretory vessels shown on left side.

NERVOUS SYSTEM

The nervous system consists of a brain, two lateral nerve cords, anterior nerves supplying the anterior region of the scolex, bothridial nerves supplying the bothridia and proboscis nerves to the proboscides.

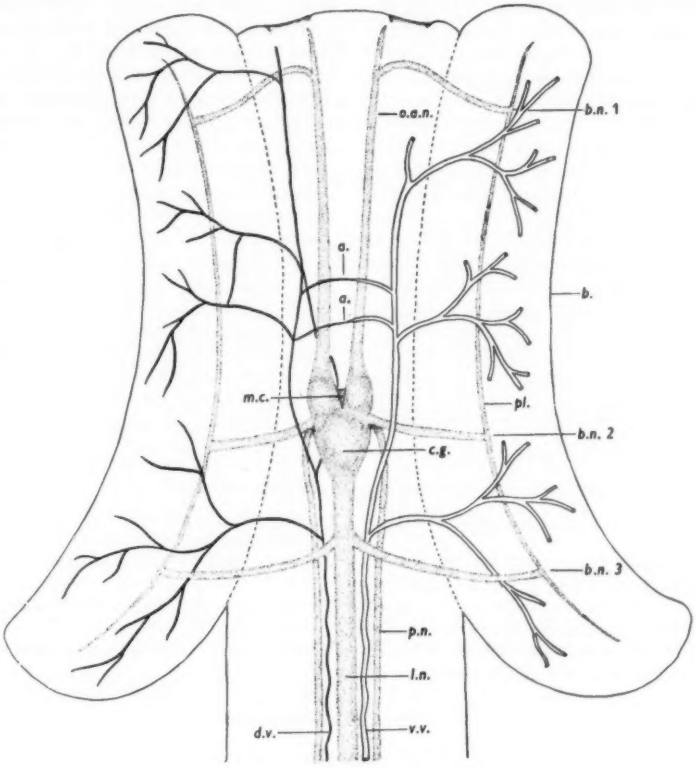
Brain. The brain is situated in the pars bothridialis at a level just behind the middle of the bothridia (Figs. 17, 18). It is compressed dorso-ventrally and elongated laterally, as it occupies a restricted area between the dorsal and ventral proboscis sheaths. The brain consists of two dorsal and two ventral ganglia, the dorsal and ventral ganglia on either side being fused (Figs. 16–18). The two dorsal and the two ventral ganglia are connected commissure is broader than the other two, being more or less spindle-shaped, the major part of it lies behind the dorsal and ventral commissures with a small portion projecting forwards between them (Fig. 17). The median commissure is the only part of the nervous system which appears to contain ganglion cells and is therefore probably the true nerve centre.

Lateral nerve cords. The lateral nerve cords arise from the fused dorsal and ventral ganglia on each side (Figs. 16–18). From their point of origin they extend obliquely outwards on either side and continue laterally throughout the body of the larva lying external to the lateral excretory vessels (Figs. 1, 11–14, 17 and 18). In the invaginated state the nerve

cords can be seen to enter the body of the larva just in front of the posterior extremities of the proboscis bulbs (Fig. 2). Here they turn forwards and continue as far as the anterior pore where they loop back again to pass to the posterior extremity.

Anterior nerves. An outer and an inner anterior nerve arise from each of the four ganglia so that there are four dorsal and four ventral anterior nerves (Figs. 16–18). The inner anterior nerves extend forwards to the tip of the scolex, curving inwards at their anterior extremities (Fig. 17). The outer anterior nerves also extend forwards, diverging slightly on either side from the inner nerves so that their extremities come to lie on the outer sides of the proboscis pores dorsally and ventrally (Figs. 17, 18). All the anterior nerves are broad nerves narrowing gradually towards their extremities.

Bothridial nerves. Three pairs of bothridial nerves enter each bothridium. The first pair arises from the outer anterior nerves near their anterior extremities. Those from the two dorsal curve round to enter the dorsal bothridium, and those from the two ventral similarly enter the ventral bothridium (Figs. 17, 18). The second pair arises from the brain itself, those entering the dorsal bothridium arise from the dorsal half of the brain, and those entering the ventral bothridium from the ventral half. The third pair of bothridial nerves arises from the lateral nerve cords a short distance behind the brain. The two dorsal again curve round to enter the dorsal bothridium, and the two ventral the ventral bothridium near their posterior margins. The bothridial nerves are connected with one another within each bothridium by a delicate nerve plexus situated about half-way along its depth (Fig. 17).



18

Fig. 18. Lateral view of brain, distribution of bothridial nerves and branches of dorsal and ventral excretory vessels.

Proboscis nerve. There are four proboscis nerves, one supplying each of the four proboscides. These arise one from each ganglion on its outer side (Figs. 16–18). The proboscis nerves pass backwards through the pars vaginalis lying between the dorsal and ventral proboscis sheaths on each side. In the pars bulbosa the proboscis nerves become applied to the

inner sides of the proboscis bulbs lying immediately in contact with the layer of myoblasts and continuing along the length of the bulbs to their extremities (Figs. 5, 8).

EXCRETORY SYSTEM

A narrow dorsal and a broad ventral excretory vessel extend throughout the body on either side. In the scolex the same vessels are present but are complicated at the anterior end by branching and anastomoses. In the entire larva the excretory vessels open into an excretory bladder which in turn opens by an excretory pore (Fig. 1). The two ventral vessels open separately, one on either side into the bladder; the two dorsal unite together immediately before they open. Lining the cavity of the bladder is a thick layer of cuticle which is frayed out into fine processes (Fig. 4), and as already mentioned the bladder is surrounded by several layers of longitudinal muscle fibres. In the body portion of the entire larva the excretory vessels take the same course as the lateral nerves. They emerge from the scolex posteriorly, turn forwards on either side, continuing as far as the anterior pore where they loop back on themselves to pass towards the posterior extremity (Fig. 2). In the scolex the dorsal and ventral vessels decrease in diameter and continue forwards unbranched as far as the pars bothridialis. A short distance behind the brain the dorsal vessel on each side divides into an inner and an outer branch. The inner branches of both sides turn inwards and pass through the nerve ring below the dorsal commissure and above the median commissure (Figs. 16–18). Some little distance in front of the brain the inner and outer branches of each dorsal vessel reunite, the outer branch having continued forwards just outside the outer anterior nerve (Figs. 16, 17). From the point of junction on each side the dorsal vessel continues forwards and joins its fellow from the opposite side across the anterior end of the scolex (Fig. 16). The ventral vessel does not divide into these two branches but continues forwards ventrally to the brain and outside the outer anterior nerve. There are two points of anastomoses between the dorsal and ventral vessels in front of the brain (Figs. 16, 17). Excretory vessels supplying the bothridia are given off from the dorsal and ventral vessels. Four bothridial branches arise from the dorsal vessel and three from the ventral vessel on each side (Fig. 18). There is an anastomosis between the second and third dorsal bothridial vessels, but no anastomosis was apparent between the ventral bothridial vessels. The bothridial vessels curve round to enter their respective bothridia in which they branch extensively.

REFERENCES

Dollfus, R. Ph. (1942). *Arch. Mus. Hist. nat., Paris*, **19**, 363–71.

Hart, J. F. (1936). *Trans. Amer. Micr. Soc.* **55**, 369–87.

Joyeux, Ch. & Baer, J. G. (1936). *Faune de France*, **30**, Cestodes.

Meischner, F. (1840). *Bericht über die Verhandlungen der Naturforsch. Gesellschaft in Basel*, **4**, 25–39.

*Oerley, L. (1885). *Termeszetrajzi fuzetek*, Budapest, 19, **2**, 97–126.

Pintner, T. (1896). *S.B. Akad. Wiss. Wien*, Mat. Nat. Classe, **105**, 652–82.

Shen, T. (1933). *J. Sci. Nat. Univ. Shantung, China*, **11**, 1–21.

Wagener, G. R. (1854). *Nova Acta Leop. Carol.* **24**, 1–91.

Wardle, R. A. (1933). *Contr. Canad. Biol. Fish.*, N.S., **8**, 77–87.

Zschokke, F. (1887). *Mitt. zool. Sta. Neapel*, **7**, 264–71.

* Not seen in the original.

Key to lettering of text-figures

All drawings are semi-diagrammatic and have been simplified for clarity.

a.	anastomosis between dorsal and ventral excretory vessels.	*m.*	muscle layer around proboscis bulbs.
a.m.	anterior muscles.	*m.b.*	muscles of wall of bulb.
b.	bothridium.	*m.c.*	median commissure.
bl.	excretory bladder.	*my.*	myoblasts.
b.n.	bothridial nerve (1–3).	*o.a.n.*	outer anterior nerve.
c.	cavity containing scolex.	*o.d.v.*	outer branch of dorsal excretory vessel.
c.g.	cerebral ganglion.	*o.m.*	oblique muscles.
c.m.	circular muscles.	*p.*	proboscis.
cu.	cuticle.	*p.b.*	proboscis bulb.
d.v.	dorsal excretory vessel.	*p.c.*	paired commissure.
e.p.	excretory pore.	*pl.*	plexus of nerves in bothridium.
i.a.n.	inner anterior nerve.	*p.n.*	proboscis nerve.
i.d.v.	inner branch of dorsal excretory vessel.	*p.s.*	proboscis sheath.
i.p.	invaginated proboscis.	*ra.m.*	radial muscles.
i.r.m.	insertion of retractor muscle on proboscis.	*r.m.*	retractor muscle of proboscis.
l.m.	longitudinal muscles.	*s.*	scolex.
l.n.	lateral nerve cord.	*t.m.*	transverse muscles.
		v.v.	ventral excretory.

(*MS. received for publication* 31. viii. 1948.—Ed.)

THE DIFFERENTIATION OF THE EGGS OF *HAEMONCHUS CONTORTUS* AND *OSTERTAGIA* SPECIES OF THE SHEEP AND A NOTE ON THE RELATIVE GENERIC EGG-LAYING RATES

By J. H. TETLEY

From the Zoology Department, Massy Agricultural College, Palmerston North, New Zealand

(With 1 Figure in the Text)

INTRODUCTION

In studying the epidemiology of nematode parasitism in sheep the collection of data in the form of populations of infective larvae cultured from the faeces of sheep involves the cumbersome procedure of incubating the material for about 9 days; further there is uncertainty of the correlation between populations found and the actual numbers of eggs in faeces from which they were derived. The problem of identifying strongyloid eggs in faeces therefore is important. Frequently, it is seen in the literature that analyses of egg counts have been made by the method of inspection, thus identification of the eggs of some species which are similar in appearance has depended much on the opinion of the particular observer. Therefore, the writer has attempted to devise a method of egg identification which would: (*a*) permit specific analysis soon after sampling, (*b*) enable compilation of a permanent record amenable to subsequent review, and (*c*) eliminate the human element. To this end the dimensional groupings of nematode eggs, paying particular attention to the cores of the specific frequency distribution, were studied. Other investigators, notably Wood (1931), Hall (quoted from Wood) and Shorb (1940), have paid attention to the dimensional range of the eggs of nematode species in sheep, but they appear to have been preoccupied more with the fact of interspecific overlap than with the intrinsic nature of the overlap.

The writer (Tetley, 1941) has shown that the eggs of *Haemonchus contortus*, when plotted lengths against widths, present a near normal frequency distribution. Applying appropriate formulae for normal frequency distributions, the writer constructed a curve embracing the statistical range means plus and minus twice the standard deviation, which is calculated to enclose 91·2 % of the population. This range was found to lie between the dimensions 72–85 by 41–48 μ. Eggs dissected from *Ostertagia circumcincta* and *O. trifurcata* (Tetley, 1941, 1949) in mixed parasitism similarly presented a distinct grouping.

Previously, in identifying eggs recovered from faeces, the writer has encountered the three genera, *Haemonchus*, *Ostertagia* and *Trichostrongylus* in mixed infection. As the egg-dimensional range of each of these genera overlaps that of the other two, the problem of identification has been difficult. The present data are uncomplicated in any significant way by *Trichostrongylus*, so that the definition of the *Haemonchus* and *Ostertagia* groupings is more clearly defined than the writer found previously

METHODS

Eggs were obtained from faeces by centrifugal flotation in a saturated common salt solution. Measurements were made by using an ocular micrometer, care being taken to support the cover-slips to prevent distortion of the eggs.

The total populations of *Haemonchus contortus*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum* were counted. Those of *Ostertagia* species, *Cooperia curticei*, *Trichostrongylus* species, *Nematodirus* species and *Strongyloides papillosus* were estimated by dilution methods, one-fifth of *Ostertagia* and one-quarter of other species being counted.

THE EXPERIMENTAL SHEEP

The experimental sheep was a 3-year-old Southdown ram, which, for a few days prior to killing, had taken little food.

EGG MEASUREMENTS

Measurements of eggs were made from faeces collected from the sheep on the day of killing. In Fig. 1, 290 eggs, selected at random for measurement,

have been plotted, lengths against widths. As the eggs of *Nematodirus* and *Strongyloides* are easily identified by inspection they were not measured.

SPECIFIC ANALYSIS OF THE EGG POPULATION

On reference to Table 1 it is seen that worms of *H. contortus*, *Ostertagia* spp., and *Cooperia curticei* considerably outnumbered those of other species found at post-mortem examination. It follows that the latter group of species must have contributed only a small proportion of the eggs found in the faeces and, therefore, were of no significance in determining the main groupings of the eggs measured. In Fig. 1 an arbitrary line, *A–B*, has been drawn between the two main groups which the writer has concluded, from references previously alluded to, and from the proportions of species found at post-mortem examination, to be, respectively, the eggs of *Haemonchus contortus* and the group *Ostertagia circumcincta* and *O. trifurcata*. A second arbitrary boundary *X–Y* based on the above-cited references separates the *Haemonchus contortus* and the *Cooperia curticei* groups.

Table 1. *The population of nematodes found in the experimental sheep*

Species	Males	Females	Total
Haemonchus contortus	204	320	524
Ostertagia spp.	5,475	6,415	11,890
O. circumcincta	3,702	—	—
O. trifurcata	1,773	—	—
Cooperia curticei	812	844	1,656
Trichostrongylus spp.	8	24	32
T. vitrinus	8	—	—
Strongyloides papillosus	0	20	20
Nematodirus spathiger	0	52	52
Bunostomum trigonocephalum	3	16	19
Chabertia ovina	6	20	26
Oesophagostumum venulosum	0	3	3
Capillaria longipes	Odd individuals		

The arbitrary boundaries must result in some *Haemonchus contortus* eggs being included in the

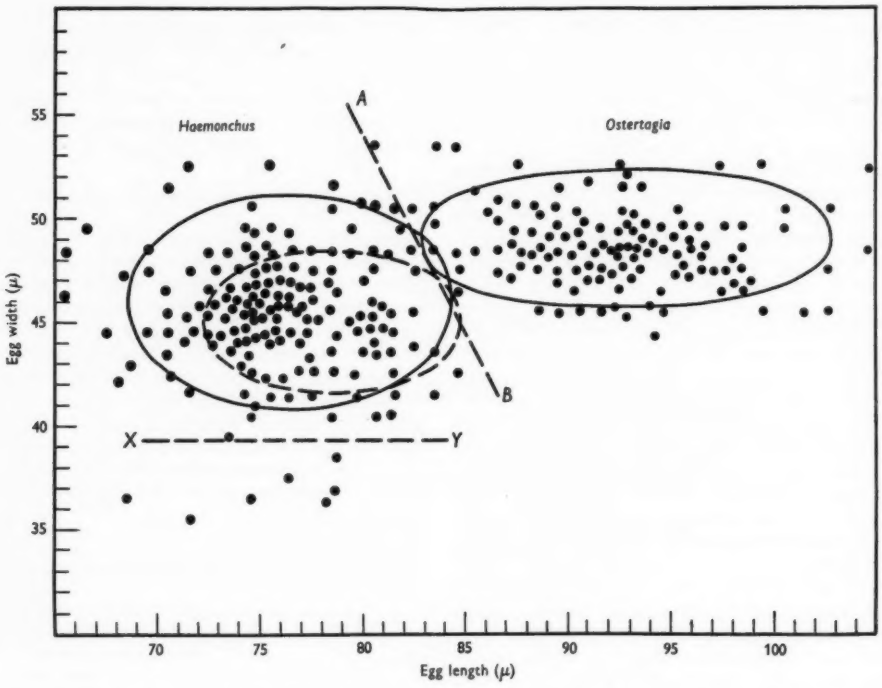


Fig. 1. The lengths plotted against widths of eggs recovered from faeces of the experimental sheep. Arbitrary boundaries *A–B* and *X–Y* have been drawn separating the egg groups, respectively, (*a*) *Haemonchus contortus* and *Ostertagia* species, and (*b*) *Haemonchus contortus* and *Cooperia curticei*. Curves calculated to enclose 91·2 % of the respective egg populations are inscribed (see text). The broken-line curve is based on data from another source (see text).

Ostertagia group and vice versa. However, as the populations of the two groups are similar, there is, to a degree, a compensating exchange of populations that reduces the small error involved in any calculations of means and statistical ranges on the arbitrary boundary basis. Errors resulting from the inclusion of *Cooperia curticei* eggs in the *Haemonchus contortus* group would also be small. Only few eggs of other species must have been present and they have been disregarded as of no significance in the present connexion.

DEVELOPMENT OF DATA

Haemonchus contortus

Those measurements falling within the zone between the lines *A–B* and *X–Y* in Fig. 1 have provided the data for the determination of the mean length and mean width of *H. contortus*. These were found to be respectively 76·43 μ (standard deviation 3·94 μ) and 45·98 μ (standard deviation 2·57 μ). The figures found by the writer (1941) for this species, in pure infection in a mature sheep, were respectively 78·49 and 44·9 μ.

The statistical range, means plus and minus twice the standard deviation, was calculated to be 68·5–84·3 by 40·8–51·1 μ. For comparative purposes the curve found for this species (1941) has been inscribed in Fig. 1, along with the curve based on present data. Probably the wider range of the curve based on the present data is due to an inherent difference in the *Haemonchus* population rather than to the presence of eggs of other species.

Ostertagia species

The second main group of egg measurement data in Fig. 1 provides the basis for calculations in regard to this genus. The mean length of the eggs of this group was found to be 92·76 μ (standard deviation 4·94 μ) and the mean width 49·02 μ (standard deviation 1·62 μ). The statistical range, means plus and minus twice the standard deviation, was found to be 82·8–102·6 by 45·7–52·2 μ. It is probable that both *O. circumcincta* and *O. trifurcata* prominently took part in determining the range of the group, as the proportions of males of these species found at post-mortem would suggest, and thus accounted for its elongate form.

THE RELATIVE EGG-LAYING RATES OF *HAEMONCHUS CONTORTUS* AND *OSTERTAGIA* SPECIES

Of the 290 eggs plotted in Fig. 1, seven are seen to fall in the *Cooperia curticei* zone. The remaining eggs fell into the *Haemonchus contortus* and the *Ostertagia* groups in the proportion of 160 to 123. By simple arithmetic the proportional egg-laying rates of members of these two groups may be calculated on the basis of the populations of females found at post-mortem examination. The result is 15·5 *Haemonchus contortus* eggs to 1 *Ostertagia* egg per individual female. Data were not available on the daily output of faeces so that it was not possible to determine the specific daily egg-laying rate.

SUMMARY

1. In a mixed infection in which *Haemonchus contortus*, *Ostertagia circumcincta* and *O. trifurcata* predominated, the lengths were plotted against widths of eggs obtained from faeces.

2. Two-dimensional groups approximating to normal frequency distributions, showing little overlap, were observed.

3. The statistical ranges of means plus and minus twice the standard deviation for the two groups barely touched. These were, to the nearest micron, 68–84 by 41–51 for *Haemonchus contortus* and, for the *Ostertagia* group, 83–103 by 46–52.

REFERENCES

SHORB, D. A. (1940). A comparative study of the eggs of various nematodes parasitic in domestic ruminants. *J. Parasit.* **24**, 223–31.

TETLEY, J. H. (1941). *Haemonchus contortus* eggs; comparison of these *in utero* with those recovered from faeces and a statistical method for identifying *H. contortus* eggs in mixed infections. *J. Parasit.* **27**, 433–63.

TETLEY, J. H. (1949). Rhythms in nematode parasitism in sheep. Parts I–VII. (In the Press.)

WOOD, W. A. (1931). A note on the size of the eggs of some species of sheep worms. *Rep. Inst. Anim. Path. Univ. Camb.* no. 2, p. 220.

(*MS. received for publication* 21. IX. 1948.—Ed.)

TWO DICHELESTHIID COPEPODS FROM MADRAS FISH

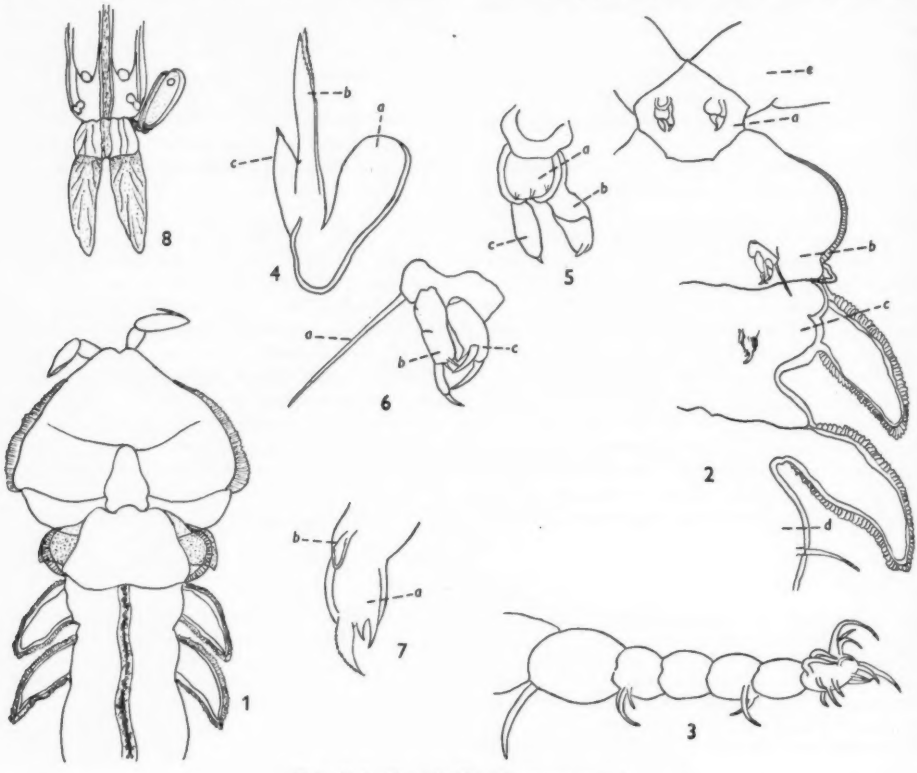
By C. P. GNANAMUTHU, *Director, Zoology Laboratory, Madras University*

(With 33 Figures in the Text)

In the course of studies of copepods parasitic on the market fishes of South India two of the most valued fishes, *Scomberomorus commersoni* and *Stromateus niger*, were found infested by large numbers of the blood-sucking parasites, *Pseudocycnus armata* Bassett-Smith and *Lernanthropus konigii* Steenstrup & Lutken. In the present paper the description of *Pseudocycnus armata*, which was first recorded by Bassett-Smith from Bombay and later by Kirtisinghe from Ceylon, is confined to a more accurate account of the appendages than has been given by these authors, while *Lernanthropus konigii*, which has not been recorded since it was established in 1861, is fully described.

Pseudocycnus armata Bassett-Smith

Host and record: Over eighty females and five males were collected from the gills of three *Scomberomorus* (cybium) *commersoni* in August 1948. The



Figs. 1–8. Female *Pseudocycnus armata*.

Fig. 1. Dorsal view of female. **Fig. 2.** Ventral view of female: *a*, first free thoracic segment and first leg; *b*, second segment and leg; *c*, third segment, leg and lateral wings; *d*, fourth segment united with trunk; *e*, bases of maxillipede. **Fig. 3.** First antenna. **Fig. 4.** First maxilla: *a*, scale; *b*, outer ramus; *c*, inner ramus. **Fig. 5.** First leg: *a*, scale; *b*, outer ramus; *c*, inner ramus. **Fig. 6.** second leg: *a*, basal spine; *b*, outer ramus; *c*, inner ramus. **Fig. 7.** Third leg: *a*, outer ramus; *b*, vestige of inner ramus. **Fig. 8.** Abdomen, anal laminae and spermatophores.

two larger fish had only female parasites, the males having probably disappeared after copulation.

Description of the female (Figs. 1–8)

Size: 7·32 mm. long (anal lamina extra), 1 mm. across cephalothorax and 1·8 mm. across the third segment.

Appendages: The first antenna is of seven joints of graded size, the basal being the largest. There are 14, 2 and 3 spines on the terminal, the third and the fifth joints (Fig. 3). The sixth joint bears a single spine which is by far the largest. The second antenna, the second maxilla and the maxillipede are as Kirtisinghe has described. The broad conical mouth-tube is chiefly formed by the lower lip, which has its anterior edges rolled up, and partly by the labrum which is shorter than the lower lip and is covered with minute spines. The mandibles are long, and taper to a slightly flattened distal part whose edges are folded up and bear about six pointed teeth on each side. The first maxilla has the complicated structure characteristic of the genus (Fig. 4). It is biramous. The basal joint bears a large elliptical scale-like plate. The inner ramus is cylindrical and ends in a sharp spine while the outer ramus is longer and ends in two elongated serrated spines. The thoracic legs are four in number. If the maxillipedes are pushed apart, the first pair of swimming legs can be distinguished easily on the narrow first thoracic segment. As these are concealed by the bases of the maxillipedes, both Bassett-Smith and Kirtisinghe missed them. They are biramous (Fig. 5). The base bears a circular spinose lamina. Both the two-jointed outer ramus and the single-jointed inner ramus bear a short terminal spine. The second leg is biramous and has a short broad base bearing a long external spine (Fig. 6). The outer ramus is two-jointed and bears a long stout terminal spine and three short spines. The inner ramus is single-jointed and bears three sharper curved spines of different sizes. The third leg is uniramous, the base bears a blunt spur, probably the vestige of the inner ramus (Fig. 7). The outer ramus is single-jointed and bears three spines of which one is large, serrated and curved, the other two being smaller. The fourth leg is as described by Kirtisinghe.

The spermatophores are elliptical plates 0·54 mm. long with rounded ends, the breadth being a third of the length (Fig. 8).

The description of the male (Figs. 9–18)

Size: 2·4 mm. long (excluding the anal lamina), 0·56 mm. broad at the cephalothorax and 0·44 mm. at the trunk.

Appendages: The first antenna is seven-jointed, each joint bears one or two spines, except the terminal which bears ten (Fig. 12). A stout curved pointed process which springs from the anterior end of the large basal joint extends to nearly its posterior limit. The second antenna is similar to that of the female, though larger relatively. The mandibles are more slender and bear only five teeth. The mouth-tube is as in the female (Fig. 11). The first maxillae are as in the female, except that the outer ramus has shorter, thinner spines. The second maxilla and maxillipedes are similar to those of the female but proportionately larger.

The swimming legs are five in number. The first is biramous; the exopod is short and conical and bears two spines on its blunt tip, while the endopod is shorter and is tipped with a spine (Fig. 13). A long curved chitinous rod, which is probably used to hook on to the cylindrical trunk of the female while the male deposits the spermatophores, springs from the base of the appendage. The second leg is biramous (Fig. 14). The base bears a long spine, the two-jointed exopod carries four stout teeth of different sizes and the endopod bears a long, curved, barbed spine beset with three smaller spines at the base. The third leg is uniramous, two-jointed, short, and conical (Fig. 15). The distal joint, which probably corresponds to the exopod, bears three spines of which the outer spine is largest and barbed. The fourth leg is as in the female (Fig. 16). The fifth leg is attached laterally about the posterior third of the trunk and consists of a short base bearing a long flagellar spine (Fig. 17).

Remarks: Bassett-Smith's creating a separate genus *Cybicola* to contain this species (*C. armata*), which he considered as not belonging to the genus *Pseudocycnus*, has been rejected by Kirtisinghe who treats this species as *P. armata* Bassett-Smith. The author of the present paper confirms Kirtisinghe's diagnosis of the species and agrees that there is no need or justification for the retention of the genus *Cybicola* instituted by Bassett-Smith.

Lernanthropus kroyeri (Steenstrup & Lutken)

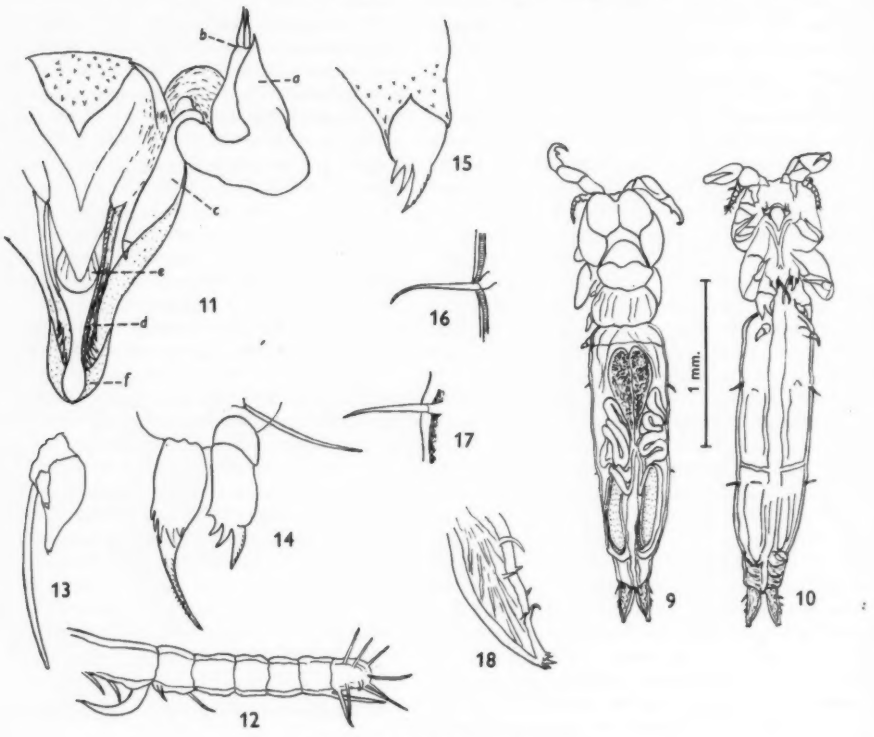
Host and record: Forty females and seventeen males were collected from the gills of five grey pomfrets (*Stromateus niger*) in August 1948. The parasites were encased in copious secretions of mucous from the host and were held between the two rows of filaments of each gill. Each parasite was attached to the distal part of a filament in such a way that only the egg strings projected beyond the gill. The body was held parallel to the filament by not only the second antennae and the maxillipedes but also by the laminate third legs behind and the cephalothoracic lobes in front.

Description of the female (Figs. 19–25)

Size: 5 mm. long, 2·1 mm. thick dorso-ventrally,

1·95 mm. broad at the middle and 0·8 mm. at the front end.

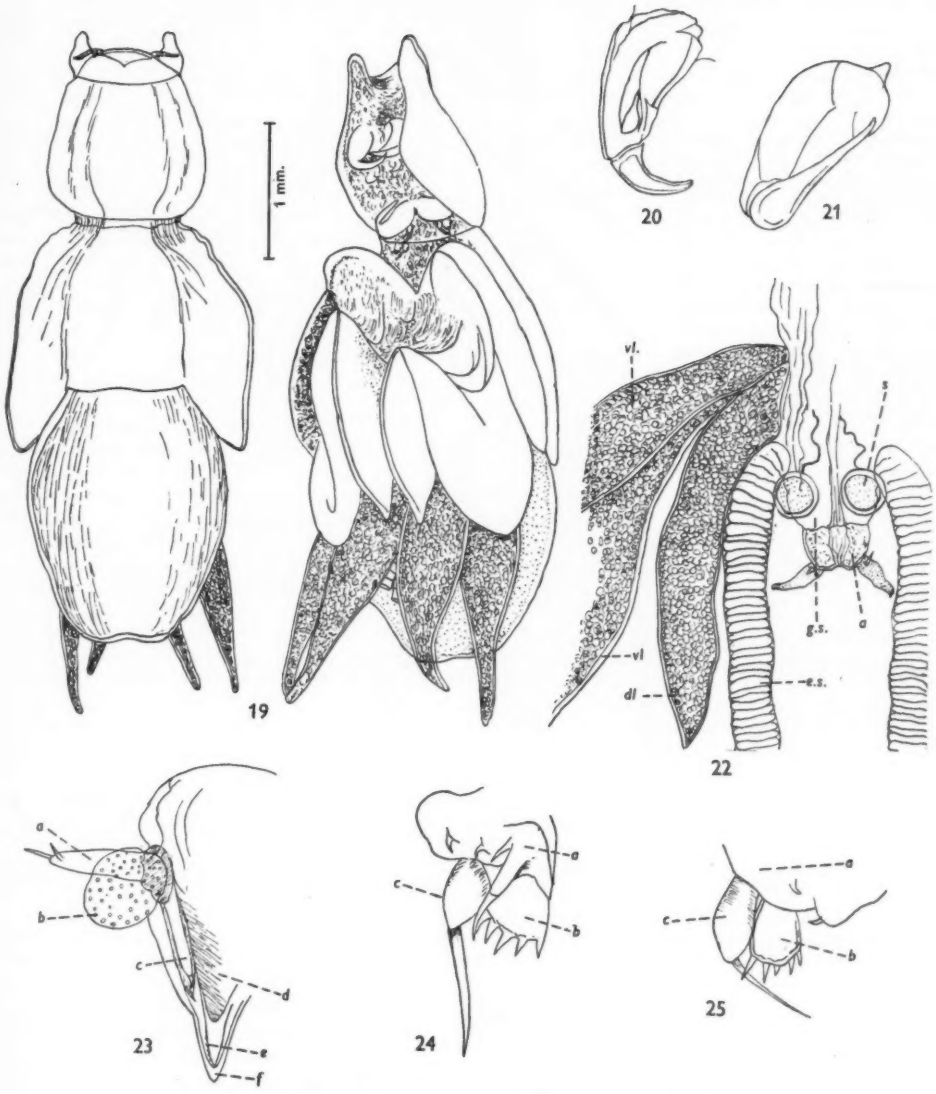
The carapace is sub-quadrangular, 1·04 mm. broad behind and 0·74 mm. in front. The lateral edges are folded down to form side flaps and the front tips of the folds are produced anteriorly beyond the front margin to form two lobes projecting forward and hold the gill filament of host between them. The frontal margin is slightly convex. Posterior to the cephalothorax, the body constricts abruptly to 0·7 mm. The trunk is covered by a dorsal plate which leaves exposed nearly a quarter of the length of the fourth legs. This dorsal plate is divided by a lateral sinus and a transverse groove (probably not visible in the preserved material examined by Steenstrup & Lutken) into an anterior plate 1·95 mm. broad and a posterior plate 1·7 mm. broad. The front plate broadens from the narrow neck and is truncated behind where it is broadest. The two hind corners are produced into lobes pressed close against the body. The hind plate is semi-elliptical, convex dorsally and has its posterior margin slightly cleft by a median shallow sinus. The anterior plate covers the first thoracic segment while the posterior plate covers the rest of the segments and the abdomen. The genital segment is very short being only 0·2 mm. long and 0·4 mm. broad. Two circular plate-like spermatophores, 0·12 mm. in diameter are invariably found attached. The abdomen is single-jointed and smaller than the genital segment, being only 0·14 mm. long and 0·2 mm. broad. The anal laminae are lanceolate and 0·16 mm. long and are sub-terminally attached to the corners of the abdomen and held divergently. Each lamina bears a long spine at the base as well as a short sub-terminal spine.



Figs. 9–18. Male *Pseudocycnus armata*.

Fig. 9. Dorsal view. Fig. 10. Ventral view. Fig. 11. Mouth-tube and first maxilla: *a*, scale of first maxilla; *b*, outer ramus of first maxilla; *c*, inner ramus of first maxilla; *d*, mandibles; *e*, upper lip; *f*, lower lip. Fig. 12. First antenna. Fig. 13. First leg. Fig. 14. Second leg. Fig. 15. Third leg. Fig. 16. Fourth leg. Fig. 17. Fifth leg. Fig. 18. Anal lamina.

Appendages: The first antennae are attached dorsal to the anterior extensions of the cephalothorax which hide them from a ventral view (Steenstrup & Lutken missed these and describe the species as lacking them). They are short, slender, seven-



Figs. 19–25. Female *Lernanthropus konigii* Steenstrup & Lutken.

Fig. 19. Dorsal and ventral views. Fig. 20. Second antenna. Fig. 21. Maxillipede. Fig. 22. Genital segment and abdomen displayed: *a*, abdomen; *dl*, lamina of the fifth leg; *e.s.*, egg sacs; *g.s.*, genital segment; *s*, spermatophores; *vl*, lamellae of the fourth leg. Fig. 23. Mouth-tube: *a*, exopod of first maxilla; *b*, scale; *c*, endopod of first maxilla; *d*, upper lip; *e*, mandible; *f*, lower lip. Fig. 24. First leg: *a*, basipod; *b*, exopod; *c*, endopod. Fig. 25. Second leg: *a*, basipod; *b*, exopod; *c*, endopod.

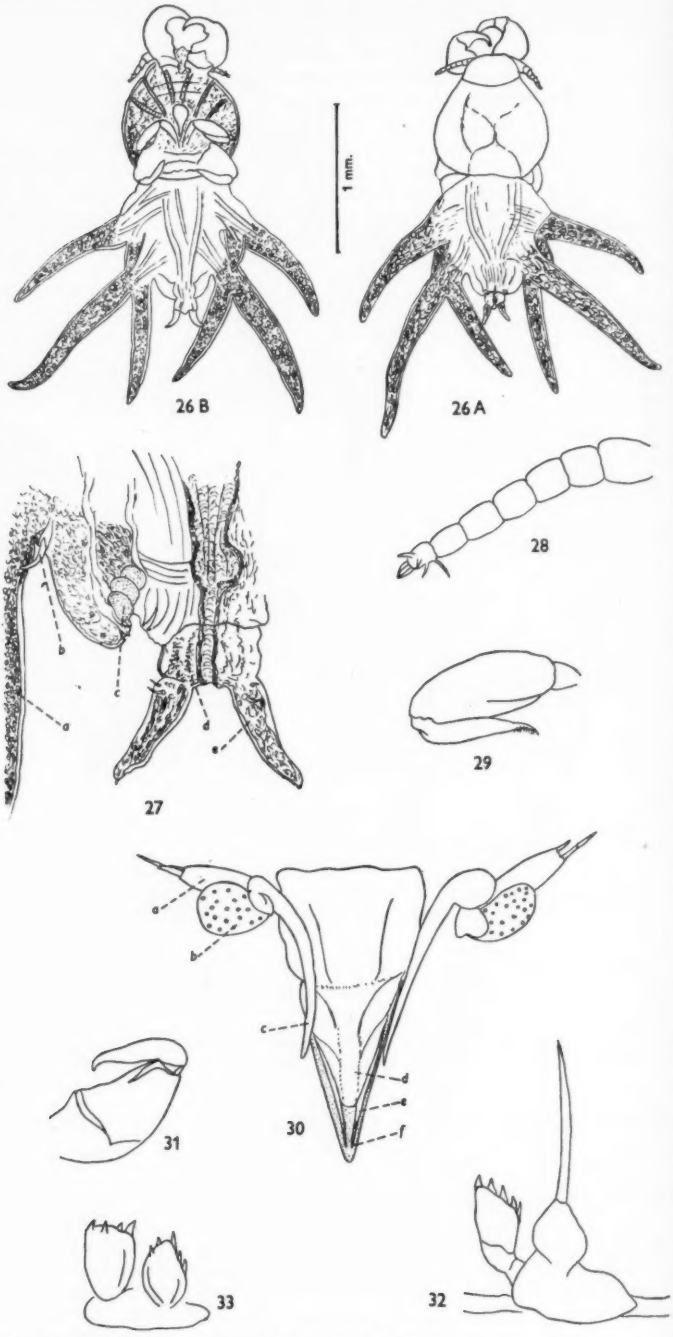


Fig. 26. A, dorsal and B, ventral views. Fig. 27. Abdomen and genital segment: *a*, lamina of fourth leg; *b*, vestigial fifth leg; *c*, spermatophores; *d*, abdomen; *e*, anal lamina. Fig. 28. First antenna. Fig. 29. Second maxilla. Fig. 30. Mouth-tube: *a*, exopod of first maxilla; *b*, scale; *c*, endopod of first maxilla; *d*, upper lip; *e*, mandible; *f*, lower lip. Fig. 31. Maxillipede. Fig. 32. First leg. Fig. 33. Second leg.

Figs. 26–33. Male *Lernanthropus konigii* Steenstrup & Lutken.

jointed and bear only three spines. The second antennae are two-jointed powerful hooks as in other species (Fig. 20). The mandibles are held within the pyriform, sharp-pointed mouth-tube formed by the larger labium and smaller labrum. Each mandible is long, jointed in the middle and toothed at its sharp distal part (Fig. 23). The first maxilla is biramous as in other species. The basal part bears a flat orbicular scale. The exopod is stout, conical and tipped by two spines, the endopod is sharp-pointed and turned towards the mouth cone and applied close to it. The second maxilla and maxillipedes are as in other species of the genus. The thoracic legs are four in number. The first is biramous and is attached close to the maxillipede on the part of the body covered by the anterior dorsal plate (Fig. 24). The base is broad and bears a long, stout spine as well as three other shorter spines. The exopod is single-jointed, there being five short spines on the outer free edge of the triangular plate. The endopod is two-jointed, a long spine being articulated to a conical base. The second leg is biramous and has a broad trilobed base bearing two small short spines (Fig. 25). The single-jointed exopod bears five short spines, while the two-jointed exopod consists of a long spine attached to a conical base. The third leg is biramous, both the rami being laminate and directed forward at an angle to the body. The endopods are applied close together round the gill filament of the host. If the body were compressed dorso-ventrally, the endopods separate as shown in the figures given by Steenstrup & Lutken. The fourth leg is bifoliate attached in front of the genital segment. Of the two rami, the outer is slightly longer than the inner which is the stouter. Another lamina which is narrower, and half as long as the others, is attached independently in front of the egg strings, to the genital segment. This probably represents the vestige of the fifth leg.

Description of the male (Figs. 26–33)

Size: Length from the frontal edge to the hind limit of the abdomen 1·8 mm., to the tips of the legs 2·5 mm. Breadth between the tips of the legs 2 mm.

The body is uncovered except for the carapace over the cephalothorax. A transverse groove marks off the antennal area of the carapace. The cephalothorax is nearly orbicular with smooth convex lateral margins and somewhat straight hind margin. The second antennae are nearly always thrust forward and the laminae of the third and fourth legs are extended outwards.

Appendages: The first antenna is seven-jointed and bears six spines on its terminal joint (Fig. 28). The second antenna is a very large, powerfully built, two-jointed claw, capable of swinging forwards. The mouth-tube is a tapering cone containing the style-like mandibles which bear minute teeth on the free tips (Fig. 30). The first maxilla is biramous, the basal part carries a flattened scale. The endopod is slender and sharp, while the exopod is conical and carries two short spines. The second maxilla and the maxillipede are of the usual form (Figs. 29, 31). There are four thoracic legs and vestiges of a fifth as well. The first is biramous (Fig. 32). The broad base carries a two-jointed endopod consisting of a long spine articulated to a short stout part, and a two-jointed exopod of a triangular form bearing five teeth on the distal edge. The second leg is of two single-jointed rami; the endopod is conical and bears five teeth, while the exopod is triangular and bears four short spines (Fig. 33). The third leg is biramous, the outer ramus is four times longer than the inner. Both rami are thin-skinned appendages which still bear a number of aborted spines. The fourth leg also is biramous, the inner ramus is only two-thirds the length of the outer. Vestiges of the fifth leg are in the form of a spine articulated to a conical base.

Remarks: Though the figures given by Steenstrup & Lutken may appear somewhat different from those given in this paper and several features of morphology presented here may not be found in the original description, yet it can be safely assumed that the present form is *Lernanthropus konigii*. The close resemblance as regards the main features of structure such as the forward lobes of cephalothorax, the dorsal plate, the form of the third and fourth legs, the laminate vestige of the fifth leg, the small genital segment and abdomen, as well as several features of the male, render the identification of the form as *L. konigii* very probable. Circumstantial evidence, such as the habitat being the gills of the same fish host (*Stromateus niger*) and the parasite occurring along with *Bomolochus megaceros*, *Synestius caliginis* and *Thysanote* (Brachiella) *appendiculata* just as noted (p. 79) by Steenstrup & Lutken, makes the probability almost certain. In conclusion *Lernanthropus konigii* may be redefined thus:

Female: The lateral edges of the cephalothorax are folded ventrally and drawn anteriorly into two forward lobes. The dorsal plate is divided transversely into two. The posterior plate covers the reduced abdomen and anal laminae but does not cover about a quarter of the fourth legs. The third legs are biramous, laminate, held at an angle. The fourth legs are bilamellate. Vestige of the fifth leg is present as a narrow shorter lamella.

Male: Smaller in size. Only the cephalothorax is covered. Third and fourth legs, biramous, foliate, the outer ramus being longer than the inner. Vestige of the fifth leg is a strong claw articulated to a basal point.

REFERENCES

BASSETT-SMITH, P. W. (1898*a*). *Ann. Mag. Nat. Hist.* ser. 7, **1**, 10–11.

BASSETT-SMITH, P. W. (1898*b*). *Ann. Mag. Nat. Hist.* ser. 7, **2**, 371.

GNANAMUTHU, C. P. (1948*a*). A new copepod parasite, *Lernanthropus dussumieria* n.sp., from the gills of a Madras fish. *Parasitology*, **39**, 209–13.

GNANAMUTHU, C. P. (1948*b*). *Lernanthropus Sciaena* n.sp. *Rec. of the Indian Museum*, Calcutta.

HELLER, C. (1868). *Reise der österreichischen Fregatte Novara*. Wien.

KIRTISINGHE, P. (1935). Parasitic copepods of fish from Ceylon. *Parasitology*, **27**, 332–44.

KIRTISINGHE, P. (1937*a*). Parasitic copepods of fish from Ceylon. II. *Parasitology*, **29**, 435–52.

KIRTISINGHE, P. (1937*b*). On the genus *Cybicola* Basett-Smith (1898). *Parasitology*, **29**, 453–6.

LEIGH SHARPE, H. (1930). *Mem. Mus. Hist. Nat. Belg.* **3**, no. 2, 1–11.

STEENSTRUP, J. J. S. & LUTKEN, C. (1861). Bidrag til Kundskab om det aabne Havs Snyltekrebs og Lernacer. *K. danske vidensk. Selsk. Skr.* **5**, 343-432.

WILSON, C. B. (1922). The Dichelesthiidae. *Proc. U.S. Nat. Mus.* **60**.

(*MS. received for publication* 17. I. 49.—Ed.)

SPERMATOPHORE PRODUCTION AND EGG-LAYING BEHAVIOUR IN *RHODNIUS PROLIXUS* STAL. (HEMIPTERA; REDUVIIDAE)

By A. KHALIFA, B.Sc. (Cairo), Ph.D. (Cantab.)
Department of Entomology, Zoological Laboratory, University of Cambridge

(With 3 Figures in the Text)

INTRODUCTION

Few hemipterans have been studied from the sperm-transference point of view. Beside the well-known case of *Cimex lectularius* L., Ludwig (1925) found in *Lygaeus equestris* L. an extraordinary long penis which reaches the opening of the spermatheca during copulation. Such a direct connexion between the penis and the female spermatheca was found to occur in another lygaeid, *Onchopeltis fasciatus* Dollas, and in the aquatic bugs *Nepa*, *Notonecta* and *Corixa*. In these forms it has been found that free sperm are delivered directly into the female organs.

In *Rhodnius* and *Triatoma*, although there is a very large aedeagus which is evaginated and fills the vagina during copulation, there is no direct connexion between it and the spermathecae. The sperm mass is deposited in the very large vagina and brought into touch with the opening of the common oviduct (the uterus of Galliard, 1935) by enclosing it in a big spermatophore which occupies the vagina till a certain amount of sperm finds its way to the spermathecae.

This paper deals mainly with *Rhodnius*. All experimental bugs were kept either singly or in pairs in 1 × 3 in. glass tubes whose open ends were covered by gauze. The culture was maintained at 27–30° C. and 70–80 % relative humidity. Blood meals were offered very frequently, within periods of 7–10 days.

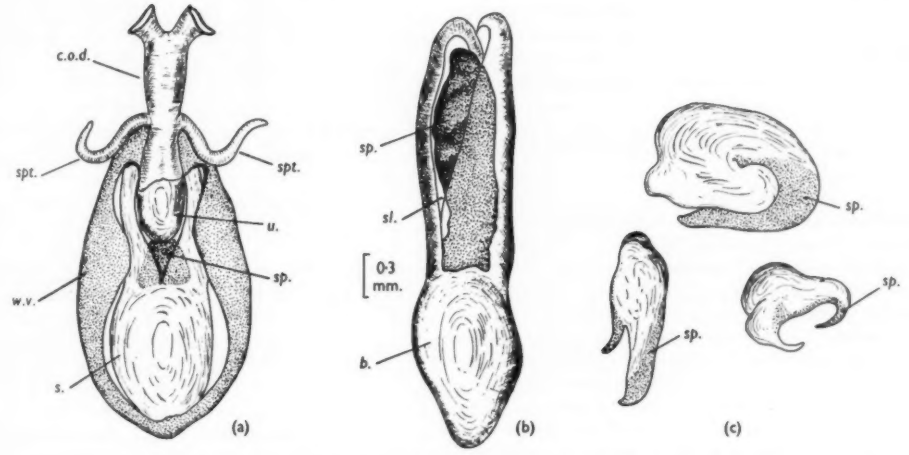


Fig. 1. (*a*) Female genital organs soon after copulation; vagina open. (*b*) Spermatophore from the vagina. (*c*) Dropped spermatophores. *b.*, solid mass; *c.o.d.*, common oviduct; *s.*, spermatophore; *sl.*, slit; *sp.*, sperm; *spt.*, spermatheca; *u.*, uterus; *w.v.*, walls of vagina.

THE STRUCTURE OF THE SPERMATOPHORE

When recently deposited it is a transparent jelly-like protein mass. It is rod-shaped, about 3 mm. long and 1·3 mm. in diameter (Fig. 1*b*). It is divisible into two parts, the anterior half, which makes contact with the opening of the common oviduct in the vagina, contains the sperm; and the posterior half which is a solid mass. The spermatophore varies considerably in size according to the amount of secretion found in the male accessory glands. When

the vagina is occupied by two or three spermatophores, they take different shapes according to the degree of crowding.

The strong muscular tip of the common oviduct which projects into the vagina, presses on the anterior half of the spermatophore and makes a slit along its dorsal wall (Fig. 1*b*, *sl.*). Through this opening direct contact between the sperm mass and the opening of the common oviduct is ensured (Fig. 1*a*). Some of the sperm is then sucked in, presumably by the muscular contractions of the common oviduct, and stored in the spermathecae. The remaining part of the sperm mass remains in the vagina and is later ejected, together with the empty collapsed spermatophore.

Every copulation results in one spermatophore, which is kept in the vagina for about 12 hr. On one occasion a spermatophore was dropped after 7 hr. from copulation. The dropped spermatophores have different forms and appear as gelatinous bodies stuck to the walls or the bottom of the container. Later, however, they dry and appear as transparent white or brownish granules (Fig. 1*c*). If any dropped spermatophore is placed in a drop of water on a slide, it becomes again soft and swells, and when examined under the microscope a smear of sperm can be seen and detached.

THE SPERMATOPHORE-PRODUCING ORGANS

The male accessory glands and the copulatory apparatus have been described by Galliard (1935), but the appearance of the accessory gland in a recent imago and in a starving male deserves further comment. There are two sets of accessory glands, each composed of four tubules and situated on one side of the abdominal cavity (Fig. 2). Each set is connected with the short ejaculatory duct by a long duct, on one side of which run the vas deferens and the vesicula seminalis. The duct of the accessory glands and of the vas deferens of each side coalesce to form a strong muscular duct which joins that of the other side at the anterior end of the ejaculatory duct.

On the first day of emergence the accessory glands contain no secretion and the vesiculae seminales contain no sperm. Three days from emergence the accessory glands contain a transparent secretion but the vesiculae seminales are still empty. On the 5th day three tubules of each set of accessory glands contain more of the transparent fluid but the fourth, which is directed towards the posterior end, contains an opaque granular secretion. The vesiculae seminales contain a great amount of sperm. This stage is reached irrespective of whether the male has been fed or not, and after about 8½ days from emergence (Table 1) the male is capable of producing the first

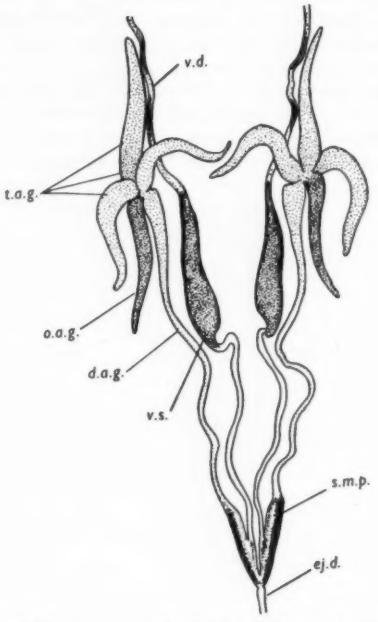


Fig. 2. Male accessory glands. *d.a.g.*, duct of accessory glands; *ej.d.*, ejaculatory duct; *o.a.g.*, opaque accessory gland; *s.m.p.*, strong muscular portion; *t.a.g.*, transparent accessory glands; *v.d.*, vas deferens; *v.s.*, vesicula seminalis.

Table 1. *Time of sexual maturity*

Male*	Date of emergence	Date of first copulation	Time in days from emergence to first copulation	Fed or not	Remarks
A	21. iv. 48	28. iv.	7	Not fed	R, 26. iv.
D	23. iv. 48	6. v.	13	Fed, 3. v.	R, 26. iv.
H	29. v. 48	6. vi.	8	Not fed	R, 31. v.
J	31. v. 48	6. vi.	6	Not fed	R, 31. v.
O	5. vi. 48	14. vi.	9	Fed, 14. vi.	R, 7. vi.
			Mean 8½		

* Every male was confined with a female in a tube since the time of emergence and the tubes were inspected every 12 hr. for the dropped spermatophores. R, refused to feed.

spermatophore. The amount of secretion in the male accessory glands was found to decrease very much when several spermatophores were produced over a short period. A decline in the size of the dropped spermatophores was also observed, the first to be produced being always the largest. The amount of sperm in the vesiculae seminales, on the other hand, was never found to undergo noticeable reduction. All dropped spermatophores without exception were smeared with some sperm. When a male whose accessory glands secretion was almost exhausted was isolated and fed, the amount of secretion in the accessory glands increased and big spermatophores were again produced. If a male was left unfed for a long time, the secretion was found to disappear gradually. In a male left unfed for 56 days the accessory glands were found very much reduced in size and contained very little secretion. The vesiculae seminales, on the other hand, were still full of sperm.

Three recently emerged males were fed once and left to starve. They were supplied with virgin females after 60, 71, and 57 days from emergence. They died on the 64th, 77th and 64th day respectively, no spermatophores were dropped and dissection of females proved that no copulation had taken place. Thus when the quantity of secretion in the male accessory gland is very much reduced by starvation, mating does not take place.

COPULATION AND EGG-LAYING BEHAVIOUR

Uribe (1927), Buxton (1930) and Galliard (1935) have given a good deal of information on the relation between feeding and the sexual life of *Rhodnius*. The number of eggs laid by mated and unmated females and also the percentage of hatching in the former have been dealt with by the same authors but not in great detail. There are, therefore, some points which need further clarification.

Anyone who has worked with *Rhodnius* will have noticed that copulation is repeatedly performed and is a common occurrence in the culture. This frequency of copulation has been found to have a bearing on the percentage of hatching of eggs. In the following set of experiments twenty-nine virgin females were used, each transferred from one tube to another every fortnight or so when the filter-paper on which the eggs were laid was almost covered with eggs. The experiment started on 26 January 1948 and was discontinued on 13 September of the same year. At the beginning of the work recently fed or starving males of unknown age were used in copulation; later, however, it became necessary to use males of known age.

The experiment was divided into five parts: in the first part eight females were used, each inseminated only once by a recently fed male of unknown age. The results for four females are shown in Table 2.

Table 2. *Egg production of females inseminated once by males of unknown age*

Female	Total no. of eggs	No. of fertilized* eggs	No. days female lived	Remarks
D	67	50 (74%)	55	Died
E	406	83 (20%)	181	Killed
H	360	50 (14%)	220	Killed
J	93	37 (40%)	40	Killed

* The unfertilized eggs retain their yellowish tinge while the fertilized ones become pink within 2–3 days, Clark (1935). Later the eyespots appear in the fertilized eggs while the unfertilized ones collapse.

The egg-laying behaviour did not differ very much in these four females. Female E will be taken as an example. She was fed for the first time in the adult stage on 26 January, copulation occurred on 3 February and the collapsed spermatophore was collected next morning. On the 9th, one egg was laid and she was fed on the same day. On the 16th, the first batch of eggs was produced and it contained thirteen eggs. In the period of 35 days from copulation she received four meals and laid eighty-six eggs, of which seventy-four hatched, nine were fertilized but did not hatch and three were unfertilized. After the end of this fertile period she started to lay unfertilized eggs and continued to do so until she was killed and dissected on 5 August. During the sterile period, of 146 days, the female received 16 meals and laid 320 unfertilized eggs. Female D was identical in general egg-laying behaviour; there was a fertile, and a subsequent sterile period and the only difference was that she did not live long. Females H and J differed from E in that they gave four and two fertilized eggs respectively during the sterile periods.

When females D and J were dissected the spermathecae were found to contain many sperm which seemed quite normal, since they moved actively in saline solution. When females E and H were dissected the spermathecae were also found to contain many sperm but they were motionless in the saline solution. The ovaries were also found to contain some eggs.

The other four females of the same group behaved quite differently. One laid a single fertilized egg that hatched while the other three laid no fertilized eggs. When the former was dissected, after 67 days during which she laid 179 eggs, the spermathecae were found to contain many sperm which appeared normal. When two of the other three females were dissected, however, no sperm could be found in the spermathecae, although copulation had occurred and spermatophores were dropped. The copulations must have been unsuccessful and for an unknown

reason the sperm could not get to the spermathecae. The dropped spermatophores were smeared with sperm as usual. The third female began to lay fertilized eggs after a further copulation.

In the second part of the experiment three females were used, each inseminated only once by a recently-emerging fed male which had not copulated before. One female lived for only 44 days and laid twenty-seven eggs and was disregarded. The second female (DD) laid eighty-four fertilized eggs and showed after that a definite sterile period. She had laid forty-three clear unfertilized eggs when the experiment was discontinued. The third female (EE) laid 139 fertilized eggs, and by the time the experiment was discontinued she did not show a clear sterile period, but her production of unfertilized eggs had increased.

there is always an excess of sperm. Although there is no quantitative evidence on the point it seemed that young males delivered more of this substance than did old ones. Towards the end of the sterile period the sperm lose their vitality completely. It is worth noting that mammalian spermatozoa contain a supplementary substance, an enzyme 'hyaluronidase', which is essential for the process of fertilization.

In the third part of the experiment six females were used, each was inseminated once by a recently fed male of unknown age and an attempt to break the sterile periods by further matings was undertaken. The egg-laying behaviour of four of them is shown in Table 3 and the graphs (Fig. 3 *a*, *b*) represent only two. It was found that a new supply of semen broke the sterile period, and renewed the output of fertilized eggs. Also, the males used in inseminating

Table 3. *Breaking the sterile periods by further copulations*

Female	Date of first copulation	Duration of fertile period (days)	No. of fertilized eggs	Duration of sterile period (days)	No. of unfertilized eggs	Date of second copulation	Duration of second fertile period (days)	No. of fertilized eggs	Total no. of eggs	Percentage of fertilized eggs
B	2. ii.	33	79	22	32	30. iii (*a*)	8	36	458 (*b*)	25
L	16. ii.	8	17	57	109	5. v (*c*)	27	97	429 (*d*)	26
N	16. ii.	3	7	98	171	31. v (*e*)	28	83	262 (*f*)	34
U	30. iii.	1	1	68	128	7. vi (*g*)	21	88	334 (*h*)	27

(*a*) Female received three spermatophores overnight, male of unknown age.
(*b*) Female lived 220 days, she was killed and dissected.
(*c*) One spermatophore was delivered, the male was 12 days old and copulated for the first time.
(*d*) Female lived 209 days, she was killed and dissected.
(*e*) One spermatophore was delivered, the male was 40 days old and this was his eighth spermatophore.
(*f*) Female lived 133 days, she died.
(*g*) One spermatophore was delivered, the male was 44 days old and this was his second spermatophore.
(*h*) Female lived 171 days, she was killed and dissected.

The conclusions which can be drawn from these observations are that: (1) a single copulation is not sufficient to ensure fertilized eggs throughout the entire life of the female. Taking females E and H of the first group as insects producing a normal full complement of eggs*, the percentage of fertilized eggs produced by a single copulation would be 20 and 14 % respectively; (2) during the early days of the sterile period the sperm, although alive and active, are unable to fertilize the eggs. I presume that by the end of the fertile period they must have consumed a certain substance which is essential for this process. This substance certainly comes from the male, but from which part is unknown: possibly from the accessory glands which contain the opaque granular secretion. This peculiar substance is delivered by the males in varying quantities, and the percentage of fertilized eggs may depend on the supply of it since females L, N, and U for the second time were young ones and the females had a higher output of fertilized eggs than when they were inseminated for the first time by males of an unknown, but certainly higher, average age.

The sterile period of one of the other two females was broken by the production of one fertilized egg only, while that of the second one did not break at all. Males of unknown age were used and one spermatophore was delivered in each case.

If the average full complement of eggs numbers 390* and the result of one copulation by a young male is the production of 98† fertilized eggs, then a female

* Uribe (1927) gives 200–300 as the total number of eggs a female can lay.

* This average was obtained from the total amount of eggs produced by females E and H of Table 2, and B, L, N and U of Table 3.

† This average was obtained from the amounts of fertilized eggs laid by DD, EE and L, N and U when they were inseminated for the second time (Table 3).

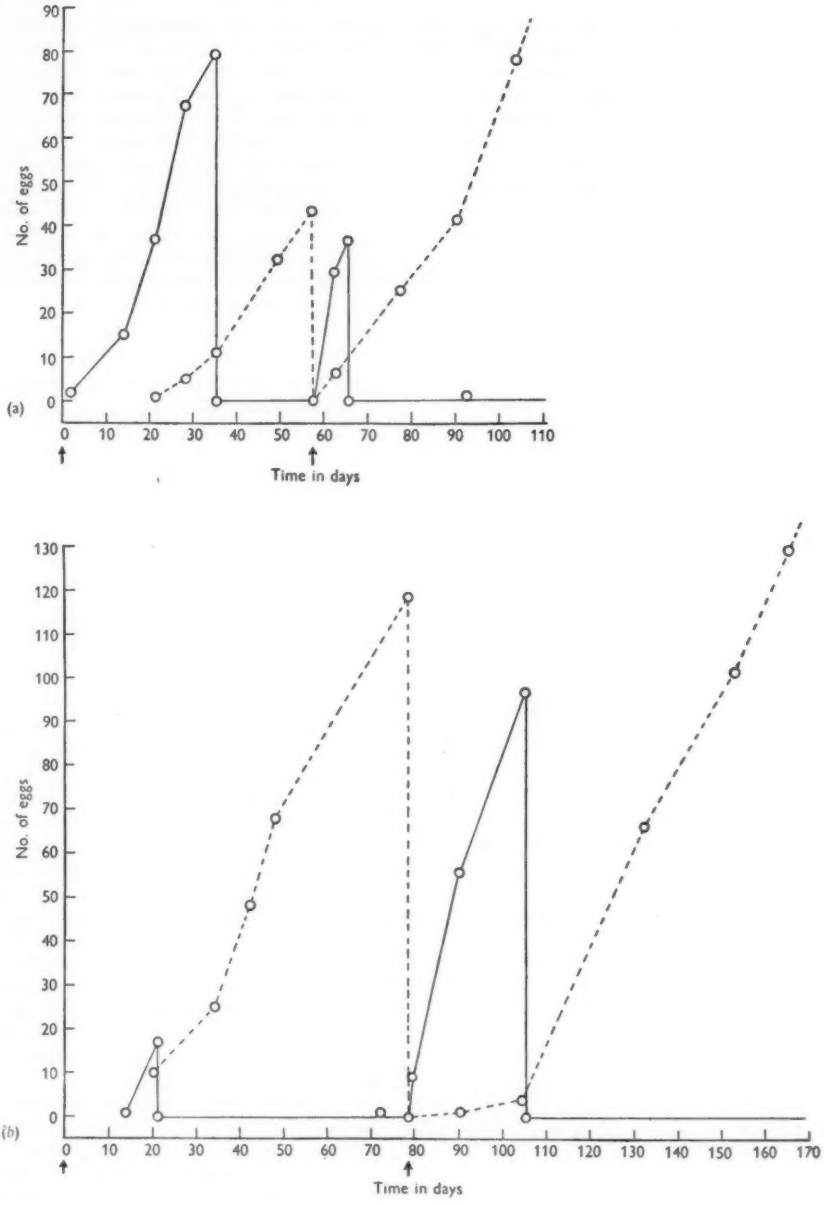


Fig. 3. The egg-laying behaviour of females inseminated twice. (*a*) Female B. (*b*) Female L (see Table 3). Unbroken lines represent the fertilized eggs. Broken lines represent the unfertilized eggs. The arrows indicate the time of matings.

should copulate four times at intervals of 45 days* with such a male if she is to produce fertilized eggs throughout her life.

Buxton (1930) found that females paired with unfed males produced fertilized eggs which were below the normal in number. He presumed that a recently fed male produced an excess of sperm which were disposed of in some way beneficial to the female. The foregoing observations on the spermatophore show how the excess of sperm is disposed of. A great number of dropped spermatophores produced by fed and starving males were examined, and they all were found to be smeared by large quantities of spermatozoa. The size of the spermathecae undoubtedly determines the amount of sperm retained by the female. It has also been shown above that spermathecae of old now-sterile females that had copulated once (females E, H and others) were found to be packed with sperm; a phenomenon which indicates that 'hypergamesis', i.e. using the excess of sperm in nutrition, never occurs in *Rhodnius*. Cragg (1923) and Abraham (1934) found evidence of hypergamesis in *Cimex*, but Mellanby (1939) did not accept their conclusions since masses of living sperm were found in the spermathecae for several weeks after copulation.

The fourth part of the experiment was designed to show if starved males were sexually less efficient than recently fed ones. Six females were used, three inseminated by males of known age and three by males of unknown age. The former were still laying fertilized eggs when the experiment was discontinued. The numbers of fertilized eggs they produced, together with those obtained from a control series of females inseminated by recently fed males are given in Tables 4 and 5.

Table 4. *Numbers of eggs produced by females paired with fed and starving recently emerged males*

	Males recently fed†		Males starved for		
			21 days	21 days	28 days
Age of males in days	9	7	35	32	32
Females‡	DD	EE	Y	GG	HH
No. of fertilized eggs	84	139	167	108	118
Mean	112·5		113		

† Males were fed and copulated on the same day.

‡ All females received one spermatophore except GG and HH which received 2. They all were left with males overnight.

This experiment shows that males starving for 21–28 days are not less efficient than recently fed ones. The numbers of fertilized eggs of the females of Table 5 are remarkably low and this is probably due to the old age of their males.

In the last part of the experiment males and females were kept continuously together to see if the fertile period can be maintained by females inseminated several times throughout their lives. Six couples were confined in tubes from the time they emerged from the last nymphal stage. Four females died very young and the other two lived till the experiment was discontinued. One of them lived 151 days and gave 412 eggs, of which 95% were fertilized and 88% hatched. She received fifty spermatophores and there was no sterile period. The second female lived 114 days and gave 253 eggs, all of which were fertilized and 98% hatched. She received eighteen spermatophores.

Table 5. *Numbers of eggs produced by females paired with fed and starving males of unknown age*

	Males recently fed					Males starved for 21 days		
Females* ...	D	E	H	J	P	K	M	T
No. of fertilized eggs	50	83	50	37	1	27	55	12
Mean	44·2					31·3		

* They all received one spermatophore except P which received 2.

* An average of 2·2 eggs per day was obtained during the fertile periods.

SUMMARY

1. Spermatophore production in *Rhodnius* and *Triatoma* is a structural adaptation for copulation and sperm delivery.

2. In *Rhodnius* a certain amount of sperm is stored in the spermathecae while the remainder is ejected with the collapsed spermatophore about 12 hr. after copulation. Hypergamesis never occurs.

3. The secretion in the male accessory glands was found to disappear if males were kept unfed for about 60 days. Such males were unable to copulate.

4. An average of ninety-eight fertilized eggs was produced as a result of one insemination by a young male. Four such inseminations at intervals of 45 days are, therefore, necessary to secure fertilized eggs throughout the entire life of the female. In nature repeated copulation is the rule.

5. The first spermatophore produced by males starved for 21–28 days was found to be as efficient as that produced by recently emerging well-fed males.

6. A male is capable of producing a great number of spermatophores; as many as fifty were obtained. Probably spermatophores produced by old males, although they contain a great number of sperm, lack sufficient of some substance necessary for fertilizing the eggs.

I am much indebted to Dr W. H. Thorpe under whose supervision this part of a Ph.D. course has been carried out, and to Dr J. S. Kennedy for reading the manuscript.

REFERENCES

ABRAHAM, R. (1934). *Z. Parasitenk.* **6**, 559–91.
BUXTON, P. A. (1930). *Trans. R. Ent. Soc. Lond.* **78**, 227–36.
CLARK, N. (1935). *J. Anim. Ecol.* **4**, 82–7.
CRAGG, F. W. (1923). *Indian J. Med. Res.* **11**, 449–73.
GALLIARD, H. (1935). Thèse fac. sci. Univ. Paris. 160 pp.
LUDWIG, W. (1925). *Z. Morph. Ökol. Tiere*, **5**, 291–380.
MELLANBY, K. (1939). *Parasitology*, **31**, 193–9.
ROTHSCHILD, L. (1947). *Brit. Med. J.* **2**, 239.
URIBE, C. (1927). *J. Parasitol.* **13**, 129–36.
WIGGLESWORTH, V. B. (1932). *Proc. Roy. Soc.* B, **111**, 364–75.
WIGGLESWORTH, V. B. (1936). *Quart. J. Micr. Sci.* **79**, 91–121.

(*MS. received for publication* 30. I. 1949.—Ed.)

MALARIA SURVEY OF THE ARAKAN REGION OF BENGAL AND BURMA

By T. T. MACAN, M.A., Ph.D.

From the Laboratory of the Freshwater Biological Association, Wray Castle, Ambleside, Westmorland

(With 2 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	290
II.	Description of the area	290
III.	Systematic notes	290
IV.	Malaria and mosquitoes in the mountain region	292
V.	Malaria and mosquitoes in the hillocks and coastal plain regions	292
VI.	Seasonal distribution of mosquitoes	294
VII.	Night observations and the study of transmission	294
VIII.	Night observations on biting time	295
IX.	Man and malaria in the Arakan	296
X.	Summary	296
	References	297

I. INTRODUCTION

The purpose of the present paper is twofold, first to put on record the findings in a part of the world previously little visited by malariologists (Senior White, 1945), and secondly to describe, and to stress the importance of, a technique of direct observation of the mosquitoes.

Although there is but a single author's name to this paper, the work was a combined effort of No. 1 Malaria Field Laboratory, Royal Army Medical Corps.

II. DESCRIPTION OF THE AREA

The area studied (Fig. 1) was a strip between the sea and the top of the first mountain range on the east side of the Bay of Bengal, and it included part of Bengal and part of the Arakan district of Burma. Three regions could be recognized: a flat coastal plain beside the sea and beside creeks which ran for some distance inland; a region of low undulating hillocks; and a mountain range rising steeply. The last is taken separately, but the first two are taken together, since the tidal creeks penetrate the hillocks in an intricate manner. In Fig. 1, distinction is made between the mountain region, indicated by diagonal lines; the uncleared part of the hillocks region, dotted; and cleared and cultivated parts of the hillocks region together with the coastal plain left white.

The coastal plain region was below sea-level at high tide, but the sea was kept out by a wall in order that the land might be cultivated. The maintenance of the sea-wall was neglected during the upheaval of war and at many places the sea water had seeped through to fill valley bottoms and other depressions. The coastal plain region was probably covered by mangrove forest before man reclaimed it.

The hillocks were covered with forest and scrub, and the flat bottoms between them were often marshy with seepages at the hill foot and open channels and pools in the marsh, but in places these bottoms had been drained, cleared, and cultivated.

The mountain region was covered with forest, and uninhabited.

Rain began to fall early in June and continued till early October and the rest of the year was dry, though showers in December were not unusual.

The people lived in houses made of bamboo, and similar structures, 'bashas', were used by the Army in back areas. Cattle were usually brought for the night into shelters which usually consisted of uprights and a roof, though some had sides.

The artificial ponds, or 'tanks', which were so numerous in the main part of Bengal, were not frequent.

III. SYSTEMATIC NOTES

Anopheles jeyporiensis. At first a distinction was made between the type form and the variety *candidiensis*, which was slightly the commoner, but, as intermediates were found, the distinction was eventually abandoned.

A. hyrcanus. All the specimens examined were var. *nigerrimus* Giles.

A. sundaicus, *A. subpictus* and *A. vagus.* Larvae

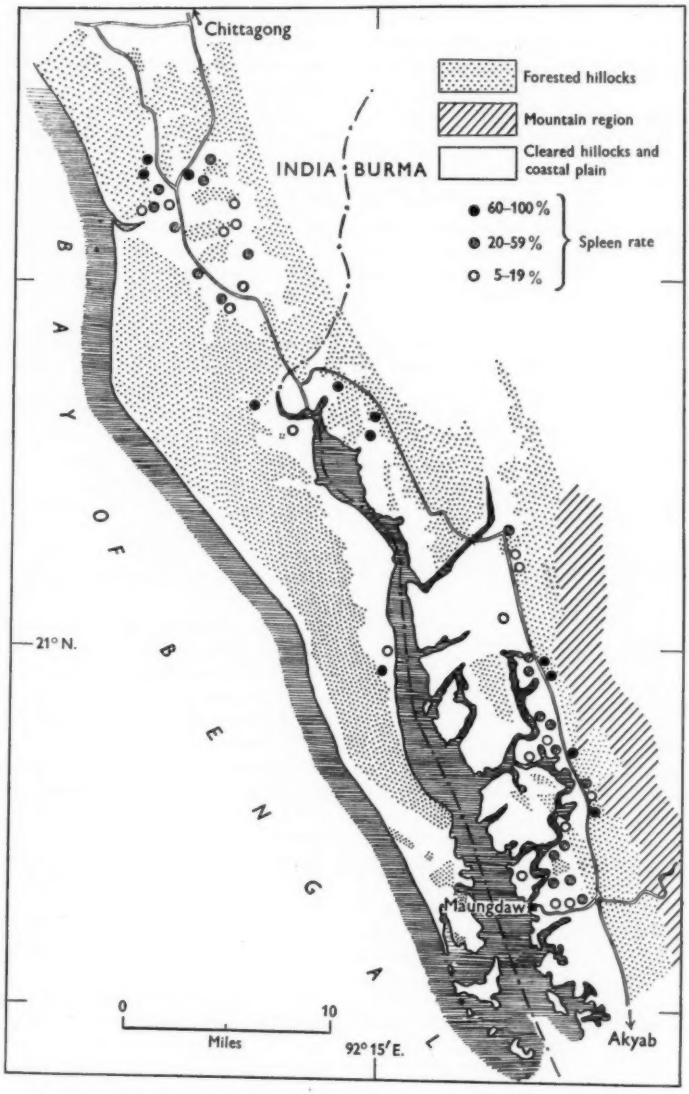


Fig. 1. Physiography and spleen rates of the area studied.

of *A. sundaicus* were invariably found together with those of *A. subpictus* and the two were always separated by breeding out the adults. No method of distinguishing the males of *A. vagus* and *A. subpictus* is known.

A. aitkeni James and *A. culicifacies* Giles, together with the species in Table 4, make a complete species list.

IV. MALARIA AND MOSQUITOES IN THE MOUNTAIN REGION

Little seems to be known about the mountain region of the Indo-Burmese border anywhere; such information as exists is discussed by Macan (1948). Table 1 shows the results obtained during the first fortnight of April. The adults were dissected and sporozoites were found in one *A. minimus*, but this species could not have been of widespread importance at this time because most of the streams were dry. *A. maculatus* was found in other streams but the adults were not biting man. No further observations were made.

Table 1. *Mosquito catches near the Ngakydauk stream, mountain region, Arakan, 31 March to 14 April* 1944

	A. minimus	*A. maculatus*	*A. barbirostris*	*A. aitkeni*
Adults caught at night in a tent	73	2	0	0
Larvae	4	42	10	7

V. MALARIA AND MOSQUITOES IN THE HILLOCKS AND COASTAL PLAIN REGIONS

Malaria was intense in many parts of these regions, but its distribution was uneven, and villages with high spleen rates were found close to villages with low spleen rates (Fig. 1).

Examination of the blood of villagers revealed active transmission in March and April, and past transmission in May and June. Dispensary figures at one place during the rains, and further blood examination after they had ceased, indicated transmission at both seasons. There was evidence of epidemic malaria, particularly in the coastal plain region.

Survey parties were in the field from March to early June, but no further observations were made till the period 17 October to 23 November.

Larval collections were made before the rains and the results are shown in Table 2.

A. karwari was found only in hill-foot seepages. A number of species were to be found in the marshy bottoms between the hillocks; *A. jeyporiensis* and *A. aconitus* were found particularly in the parts thickly overgrown with vegetation, often where standing water was not visible till a pool gathered round the collector's feet. *A. vagus* was found in small pools and borrow-pits with vegetation. *A. sundaicus* and *A. subpictus* were the species of the coastal plain region, where they bred in borrow-pits and places flooded owing to neglect of the sea-wall.

Adult mosquitoes were captured by day in the usual way, and also while they were active at night. Different places varied in their suitability for this purpose. The best was a settlement of brick-makers in a series of bashas at Chunati near Chiringa, for they had no women and were far from the fighting, so that there was no objection to collectors going everywhere at night. The worst were front-line positions, where the collector was confined to a dug-out in which he sat in the dark until he heard a mosquito, when he pulled a black-out curtain over his hole and then tried to catch the mosquito. The human population being spread over a wide area, the number of mosquitoes coming to one dug-out was not likely to be large. In villages one house was occupied for the night and men were hired to sleep in it as bait; since the people were Moslems, it was not possible to wander all through the village at night as in a Buddhist village.

Table 3 shows the adult catches at Chunati, the only place to yield big catches, and it illustrates clearly the importance of catching mosquitoes at night, which subject, however, is discussed fully later. It is also interesting to compare the catch made near the village in the forest using three men as bait with the catch made inside the bashas. *A. minimus* did not attack in the forest, *A. barbirostris* did not attack indoors, and more *A. kochi* and *A. leucosphyrus* attacked outside than inside.

Table 4, line 2, shows the catches made in the rest of the area, and the striking point about them is their small size. Ten out of the fourteen were in front-line positions where, as explained, small catches were to be expected, although there were well-occupied breeding places nearby. In many parts of both regions mosquitoes are likely to be at a minimum at this time when the water-table is at its lowest just before the rains. In the four villages spleen-rates ranged from 66 to 100%, and the parasite examination showed evidence of transmission not long ceased, so there must have been a good population of mosquitoes in the recent past. A single night catch near cattle yielded 136 *A. subpictus*, 21 *A. sundaicus* and 1 *A. aconitus*. Day catches are not worth recording as they yielded little except *A. subpictus*, *A. vagus*, and *A. sundaicus*, though at one place 2 specimens of *A. culicifacies* were taken. Since this was our only record of this species, we were puzzled by the importance which Grewal (1937) attaches to it.

Line 3 of Table 4 shows comparable catches in October, line 4 night catches near cattle in October, and line 5 the results of an intensive night-catching

Table 2. *Larval catches, March to June 1944*

	A. jamesi	*A. subpictus*	*A. vagus*	*A. aconitus*	*A. sundaicus*	*A. jeyporiensis*	*A. barbirostris*	*A. annularis*	*A. hyrcanus*	*A. philippinensis*	*A. karwari*	*A. maculatus*	*A. minimus*
Total no.	149	96	93	73	58	56	48	41	23	20	18	10	2
No. of breeding places	22	24	17	12	9	13	13	14	9	5	3	3	2

Table 3. *Adult mosquitoes caught at Chunati brick-field, near Chiringa*

Date	Type of catch	No. of observations	*A. jeyporiensis*	*A. philippinensis*	*A. minimus*	*A. aconitus*	*A. annularis*	*A. vagus*	*A. subpictus*	*A. vagus* and *A. subpictus* males	*A. kochi*	*A. barbirostris*	*A. karwari*	*A. leucosphyrus*	*A. jamesi*	*A. varuna*	*A. sundaicus*	*A. maculatus*	*A. hyrcanus*	*A. ramsayi*	*A. tessellatus*	*A. pallidus*
April 8	Day catch in bashas	1	3	—	6	2	—	5	1	14	—	—	—	—	—	—	—	—	—	—	—	—
4/5 April	Night catch in forest	1	5	3	—	2	—	—	—	—	6	5	2	4	—	—	—	—	—	—	—	—
5/6, 8/9 April	Night catch in bashas	2	118	57	34	6	10	—	—	—	2	—	2	5	2	1	—	—	1	—	—	—

Table 4. *Summary of total mosquitoes caught in night catches in hillocks and coastal region of Arakan*

Date	Place and details	No. of collections	*A. jeyporiensis* James	*A. philippinensis* Ludlow	*A. minimus* Theobald	*A. aconitus* Dönitz	*A. annularis* Van der Wulp	*A. vagus* Dönitz	*A. subpictus* Grassi	*A. vagus* and *A. subpictus* males	*A. kochi* Dönitz	*A. barbirostris* Van der Wulp	*A. karwari* James	*A. leucosphyrus* Dönitz	*A. jamesi* Theobald	*A. varuna* Iyengar	*A. sundaicus* Rodenwaldt	*A. maculatus* Theobald	*A. hyrcanus* Pallas	*A. ramsayi* Covell	*A. tessellatus* Theobald
April	Chiringa, indoors	2	118	57	34	6	10	—	—	—	2	—	2	5	2	1	—	—	1	—	—
April to May	All stations with human bait, excluding Chiringa	14	9	—	2	3	—	23	29	6	—	1	—	—	1	—	9	—	—	—	—
October to November	Stations with human bait	15	15	4	8	39	7	—	8	—	—	—	—	15	—	—	108	—	24	—	—
October to November	Stations with cattle bait	5	1	3	—	34	20	18	13	2	—	8	1	—	11	—	11	1	54	—	—
November 13 to 23	All stations during course of DDT experiment	50	1	14	4	586	159	147	1398	—	—	20	17	1	237	309	1071	1	225	1	1

Table 5. *Comparison of the total number of larvae and the total number of adults near man at night in the period before the rains*

	A. jeyporiensis	*A. philippinensis*	*A. minimus*	*A. leucosphyrus*	*A. kochi*	*A. sundaicus*	*A. subpictus*	*A. jamesi*	*A. vagus*	*A. aconitus*	*A. barbirostris*	*A. annularis*	*A. hyrcanus*	*A. karwari*	*A. maculatus*
Larvae	56	20	2	0	0	58	96	149	93	73	48	41	23	18	10
Adults	132	60	36	9	8	9	29	3	23	11	6	10	1	4	0

Table 6. *Adult catches in October and November*

Place	Time	*A. sundaicus*	*A. leucosphyrus*	*A. jeyporiensis*	*A. minimus*	*A. philippinensis*	*A. hyrcanus*	*A. aconitus*	*A. annularis*	*A. subpictus*	*A. vagus*	*A. vagus* and *A. subpictus* males	*A. pallidus*	*A. barbirostris*	*A. jamesi*	*A. maculatus*	*A. karwari*
Houses and tents	Night	108	15	15	8	4	24	39	7	8	—	—	—	—	—	—	—
Houses and tents	Entering at dawn	—	—	—	—	—	2	—	—	5	38	26	—	—	—	—	—
Houses and tents	Day	22	—	1	1	—	—	65	14	1	47	18	—	18	1	—	1
Near cattle	Night	11	—	1	—	3	54	34	20	13	18	2	1	8	11	1	1

programme, with several collectors and several types of catching place, during the course of an airspray experiment with DDT.

No larval collections were made after the rains, so this concludes the presentation of the data.

VI. SEASONAL DISTRIBUTION OF MOSQUITOES

From work of this type, in which few places were visited more than once, only the main trends of seasonal abundance can be deduced. *A. jeyporiensis* was the commonest species at Chunati in April (Table 3) and fair larval populations were found in other parts in April and May (Table 2). At Chunati in late May it was much scarcer, only a few adults being taken, and it appears therefore to be a species of the early months of the year. *A. minimus* was fairly widespread after the rains (Table 4) but the numbers were unexpectedly small. Anti-malaria unit records showed that *A. sundaicus* was abundant late in the year at places where we found it scarce before the rains. We gained the impression that its numbers were liable to sudden local fluctuations. Thus larvae were present in great numbers in a borrow-pit after this had been filled by a spring tide, but a second inundation brought fish and the numbers declined suddenly. *A. hyrcanus* was more abundant after the rains. The impression on the minds of some colleagues who lost their data is that larvae of *A. barbirostris* were more abundant in March than in the subsequent months.

VII. NIGHT OBSERVATIONS AND THE STUDY OF TRANSMISSION

Search for larvae was fairly thorough and the figures in Table 2 probably give a rough indication of the proportion of different species in the area. It is interesting to compare with them the figures for mosquitoes caught near man (Table 5). The first five species are evidently attracted to man, whereas the rest presumably prefer some other animal. Further analysis of night observations is possible. Some results have already been noted in Table 3, but the most thorough set of observations was made by Major G. I. Watson in October. His findings are shown in Table 6 and graphically in Fig. 2.

From these results it is possible to make several important deductions. Four of the species, *A. sundaicus*, *A. leucosphyrus*, *A. jeyporiensis* and *A. minimus*, were feeding almost exclusively on man, since it was in the neighbourhood of human beings that they were found at night. *A. philippinensis* was attacking both man and cattle, though the figures are not sufficiently large to justify any statement about the extent to which it was feeding on the one rather than on the other. Though twice as many *A. hyrcanus* were found attacking cattle as were found attacking man, nevertheless a significant number were feeding on man. Twenty-three out of the twenty-four were, however, found in one camp where there were no cattle, which suggests that this species is primarily a cattle-feeder but will readily turn to man if cattle are not available. *A. aconitus* was taken near man at night but examination showed that most of the specimens, which were taken mainly in tents, had old blood in the abdomen, and so had presumably fed a night or two previously and were merely seeking shelter. Nearly all the freshly fed specimens of *A. aconitus* were taken near cattle. *A. annularis*, *A. subpictus*, *A. vagus*, *A. barbirostris* and *A. jamesi* were feeding mainly or entirely on cattle.

The species found resting in houses by day were those which were biting cattle by night, and all the cattle-feeders except *A. hyrcanus* and *A. jamesi* were house-resters. On the other hand, the man-biters, which were taken in houses by night, were only rarely taken indoors by day. *A. vagus* and *A. subpictus* entered houses at dawn, a phenomenon noticed elsewhere on many occasions. Nothing was discovered about the resting-places of those species which were not found in houses by day.

It may be deduced that *A. jeyporiensis*, *A. minimus* and *A. leucosphyrus* are the important vectors, if they attain sufficient abundance, a point established only for the first-named (Table 3); that *A. sundaicus* is also important, though findings elsewhere showed that it was deflected to cattle more than these figures suggest; that *A. philippinensis* must be suspected, having regard to the findings at Chunati (Table 3); and that *A. hyrcanus* and *A. annularis* may be important locally under war conditions. This circumstantial evidence is, it is suggested, of real value. Unfortunately confirmation was obtained in one instance only, but it is significant that the three species most suspected are all noted vectors, and the rest are known to carry malaria in some parts of their range.

The salivary glands of all the specimens collected (Table 3) at Chunati were examined and sporozoites were found in two specimens of *A. jeyporiensis* (1·6 %). Circumstantial evidence against this species was found at two other places. Thus around Tumbru in March it was the only species found in larval collections near each of six villages where blood examinations showed that active transmission was in progress; unfortunately at that time the importance of night catches had not been discovered. adults were sought only by day, and few were taken. In the Razabil area catches were low, for reasons already discussed, but *A. jeyporiensis* was the commonest species encountered and one battalion, in whose positions observations were made, was showing at that time a rate of malaria higher than that of any other unit in the area. The conclusion

reached was that most of the pre-monsoon malaria in the hillocks region of the Arakan was transmitted by *A. jeyporiensis*.

A. minimus is a notorious vector throughout its range and is likely, therefore, to play some part in transmission in the Arakan but, since, except at Chunati, it was taken only in small numbers, the part may not be a major one.

A. leucosphyrus is a vector in the forests of central Burma during the rains (Macan 1948), and may be a vector under similar conditions in the Arakan, but, as no observer was present during the rains, it is not possible to say whether or not this species was abundant then.

A. sundaicus invaded the hillocks area in considerable numbers at certain places. From the Tumbru region 290 specimens were dissected in May and early June, but no sporozoites were found. This, however, is not unexpected, since many of the specimens were collected in cattle shelters before the extent to which *A. sundaicus* was deviated to cattle had been discovered. When a night catch was made in a village with cattle stabled between the houses and the breeding place it was found that very few *A. sundaicus* penetrated past the cattle to feed on humans.

Malaria occurred in villages which lay in the coastal region well away from any other region and, since under similar conditions elsewhere, *A. sundaicus* has been shown to be the vector, it probably transmits also in the Arakan. There was evidence that, as in other localities, it tended to cause epidemics, often within quite a small area, at irregular intervals, and was not responsible for steady or widespread transmission every season.

Between 28 June and 7 July some observations were made in the Sundarbuns where conditions are similar to those of the Arakan except that much of the mangrove forest is untouched and clearings occur only at intervals. I am informed by Dr Iyengar that *A. sundaicus* is not found in the untouched mangrove jungle and this statement was confirmed by limited observations. *A. sundaicus* was abundant and active in the clearings, and catches made on coolies during six consecutive nights were 308 on six coolies, 138 on three, 216 on three, 252 on three, 103 on 2, and 146 on two. From these catches 534 specimens were dissected and 7 had sporozoites (1·3%).

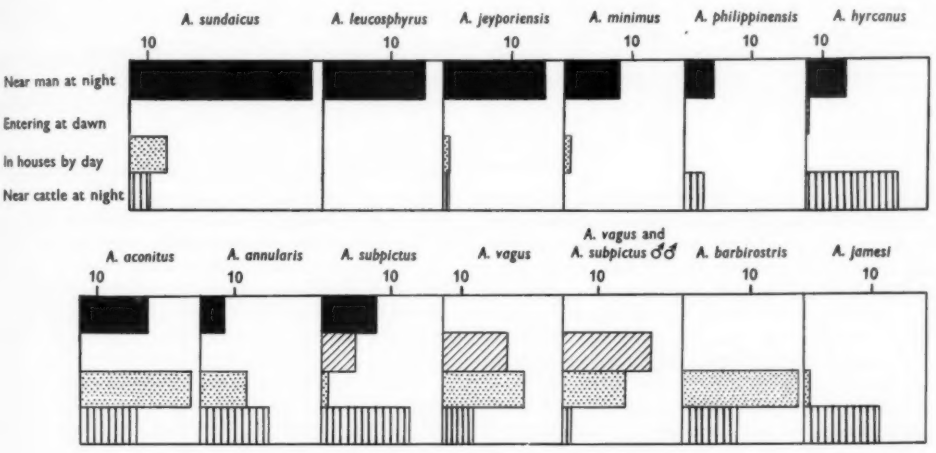


Fig. 2. Feeding and resting places of species of *Anopheles*.

VIII. NIGHT OBSERVATIONS ON BITING TIME

At Chunati in April there were three common species and consequently precise observations on any one were not possible. Mosquitoes appeared in the bashas between 22.00 hr. and midnight, most fed between 01.30 and 03.30 hr., and then they gradually disappeared. In a room occupied by sick men, who neither smoked nor cooked as the others did, the whole course of events was earlier and some fed mosquitoes were found between 21.00 and 21.30 hr.

More accurate observations were made in May during the course of repellent experiments at Chunati. It was found then that there was some biting at 22.30 hr. when the experiments usually started, that the numbers of *A. philippinensis* showed a small peak round about midnight and a main peak between 03.00 and 04.00 hr., and that *A. minimus* had a single peak biting-period between 03.00 and 04.00 hr. Another important observation

was that *A. minimus* entered the houses, whereas *A. philippinensis*, when it was not raining, tended to attack the people sleeping out of doors, a habit noted elsewhere by Macan (1948, p. 258), who records that no specimens were found resting nearby before or after feeding.

The catches made during the experiments with DDT were separated into two categories, specimens taken before and after 01.00 hr. (Table 7).

Table 7. *Biting times observed in November*

	A. sundaicus	*A. subpictus*	*A. aconitus*	*A. varuna*	*A. jamesi*	*A. hyrcanus*	*A. annularis*	*A. vagus*
Before 01.00 hr.	290	431	217	60	63	41	33	36
After 01.00 hr.	328	330	167	77	59	65	49	33

Observations on the biting-time of *A. sundaicus* were made during the Sundarbans excursion, where continuous catches of mosquitoes attacking hired coolies went on throughout the night. Although unfed females began approaching human bait soon after sunset there was little biting before 21.00 hr., though occasionally an odd specimen attacked earlier, and after that time mosquitoes attacked till dawn. They came in waves, the first just before midnight and three between midnight and dawn. The peak before midnight was invariably the smallest and the third was usually the largest, but on two out of six nights the greatest intensity of biting was recorded just before dawn. On one occasion all the personnel had been protected by impregnated clothing, and it was noted that several specimens, baulked of a feed during the hours of darkness, waited long after sunrise till the removal of the clothing gave them a chance to attack. One was caught feeding on the open fore deck of a ship going at full-speed ahead more than an hour after sunrise. At night *A. sundaicus* was little deterred by heavy rain or by a fairly strong wind; when the wind was blowing, it always found a sheltered place on the lee side of the body before it attempted to bite. Other things being equal it usually chose a site an inch or two from the ground.

If a daylight attack by specimens baulked of a feed at night is a widespread habit, it could be important, for it could cause much malaria among a community which observed with care the regulations about wearing long sleeves and trousers after dusk and the proper use of the mosquito net. That *A. philippinensis*, under certain circumstances, attacks people out of doors without resting near them is another important observation, because by doing this it avoids surfaces treated with DDT and other contact insecticides. Neither of these observations would have been made but for the practice of catching mosquitoes by night. This gave, furthermore, a reliable picture of the mosquito population and provided strong circumstantial evidence about which species were vectors. Day catches, on the other hand, gave a false picture of the important mosquito population (Table 3, Table 6 or Fig. 2), and might indeed fail to secure the vector altogether. Day catching was still the standard teaching when these observations were made. Results suggest that, in some parts of the world at least, it is of no value.

IX. MAN AND MALARIA IN THE ARAKAN

The foothill region in its natural state is potentially highly malarious. Breeding facilities for several vector species are extensive, mainly in the ill-drained valley bottoms between the hillocks. When a human community settles down, clears the jungle, and terraces the valley bottom for rice cultivation, it changes conditions in such a way that unimportant species of *Anopheles* become commonest and the amount of malaria transmission is reduced. In the flat rice-growing area of Bengal to the north-west of the Arakan, spleen rates seldom exceed 20% and there can be no doubt that cultivation in the foothill region of the Arakan reduces the amount of malaria though it does not banish it completely.

In the coastal areas the reverse is true. In its virgin state the intertidal zone is covered with mangrove forest and is malaria-free. Man has removed the forest, and reclaimed the land by building sea-walls. Invariably suitable brackish breeding-places come into being as a result of these operations, *A. sundaicus* establishes itself, and malaria follows.

X. SUMMARY

1. The area (Fig. 1) may be divided into a forested uninhabited mountain region, a region of low hillocks with between them marshy bottoms, many of which had been cleared and cultivated, and a flat coastal plain protected from the sea by a wall which leaked. Rain fell from early June till late September, and the rest of the year was mainly rainless.

2. Little is known about the mountain region. *A. minimus* was found infected in April, at which time, however, most potential breeding-places were dry. Larvae of *A. maculatus* were abundant, but the adults did not bite man.

3. Malaria was intense locally in the hillocks and coastal plain region, but uneven in distribution (Fig. 1). Evidence of active transmission in March and April, during the rains, and in October was found, there was a lull in May and June, but any other lulls there may have been were not discovered, since observations through the year were not possible.

4. Observations at night revealed *A. leucosphyrus*, *A. jeyporiensis* and *A. minimus* near man and rarely near cattle. *A. sundaicus*, *A. philippinensis*, *A. annularis* and *A. hyrcanus* attacked both man and cattle, though some, at least, would attack man only in the absence of cattle. *A. aconitus* was found near man at night, but nearly all the specimens contained old blood.

5. *A. subpictus* and *A. vagus* fed on cattle and entered houses at dawn in search of shelter for the day. The man-biters mainly sought shelter in some outdoor resting-place. The conclusion was that the standard practice of searching for mosquitoes in houses early in the morning gave results which were not only valueless, but misleading (Fig. 2).

6. Few dissections were made and sporozoites were found only in *A. jeyporiensis* (2/126 = 1·6 %), which was thought to be responsible for most of the malaria transmitted early in the year. It is suggested that the results of night catching provide valuable circumstantial evidence about vectors.

7. The night catches also gave information about time of biting, and yielded two observations of importance in control work. *A. minimus*, *A. jeyporiensis*, *A. philippinensis* and *A. sundaicus* did not attack until several hours after nightfall. *A. philippinensis*, in late May, when it was not raining, preferred to bite persons sleeping out of doors. Elsewhere it was observed that *A. philippinensis* did this and also did not rest nearby before or after feeding as other species did. It might, therefore, avoid a contact insecticide. *A. sundaicus*, baulked of a feed by night, attacked after sunrise.

REFERENCES

GREWAL, R. S. (1937). Notes on the anopheline mosquitoes of villages on the Arakan coast of Burma. *Rec. Malar. Surv. India*, **7**, 267–8.

MACAN, T. T. (1948). Mosquitoes and malaria in the Kabaw and Kale valleys, Burma. *Bull. Ent. Res.* **39**, 237–68.

SENIOR WHITE, R. (1945). Some landmarks and lacunae in Malariology in Bengal. *Bengal Publ. Hlth. J.* **3**, 39–44.

(*MS. received for publication* 18. II. 1949.—Ed.)

NOTES ON PARASITIC MITES. I

SOME REMARKS ON THE FAMILY LAELAPTIDAE (*SENSU* VITZTHUM 1943) WITH DESCRIPTIONS OF THREE NEW SPECIES FROM AFRICAN RODENTS

By F. ZUMPT, Ph.D.

The South African Institute for Medical Research, Johannesburg

(With 4 Figures in the Text)

The systematic position of the blood-sucking Gamasides Leach, 1815, is still very unsatisfactory. In his work on external parasites, Ewing (1929) recognizes the two families Parasitidae and Dermanyssidae, distinguishing them chiefly by the form of the chelicerae. The former family to which *Laelaps* and related genera belong is defined by Ewing as follows: 'chelicerae of a generalized type having the tips incurved and being provided with teeth, and the fixed arm usually bearing a seta near its tip'. The Dermanyssidae have modified chelicerae, 'usually without teeth and fixed arm always without setae'. In this family Ewing puts the genera *Liponyssus*, *Dermanyssus* and related ones.

It is clear from these characters that there is no very distinct difference between the two families. It has happened more than once that a new species placed in the Parasitidae has later had to be transferred to the Dermanyssidae, and vice versa. Studying the mouthparts of the different genera of these families, all transitions from the primitive biting chelicerae of *Laelaps* or *Hypoaspis* to the more specialized piercing organs of *Dermanyssus* may be seen. Correlated with this transformation of the chelicerae is a reduction of the body sclerotization which enables the mites to take more blood or lymph at one meal. Furthermore, the tendency to a closer and more distinct parasitic life leading from an ectoparasitism to an endoparasitism (for instance, *Entonyssus* and *Pneumonyssus*) is to be noted.

Vitzthum (1943), therefore, in his last comprehensive work on the Acarina has united these two families under the name: Laelaptidae. He divides them into thirteen subfamilies in the order given below:

(1) Hypoaspidinae Vitzthum n.sub.—free living, or not very much modified ectoparasites, females incline to symphoristic habits.

(2) Podocininae Berlese, 1916—free living, rarely symphoristic.

(3) Hyletastinae Vitzthum n.sub.—free living, females incline to symphoristic habits.

(4) Iphiopsinae Kramer, 1886—ectoparasites of insects and paraphages of myriapods.

(5) Phytoseiinae Berlese, 1916—free living.

(6) Halarachninae Oudemans, 1906—endoparasites in lungs of mammals and nose-cavities of seals.

(7) Haemogamasinae Oudemans, 1926—ectoparasites on small mammals.

(8) Laelaptinae Tragardh, 1908—ectoparasites on small mammals, partly perhaps only paraphages.

(9) Dermanyssinae Kolenati, 1859—ectoparasites on mammals and birds with strongly modified chelicerae.

(10) Liponyssinae Ewing, 1923—ectoparasites on vertebrates.

(11) Entonyssinae Ewing, 1923—endoparasites in lungs of snakes.

(12) Raillietiinae Vitzthum n.sub.—parasites in the ears of ungulates.

(13) Rhinonyssinae Trouessart, 1895—endoparasites in the nose-cavities of birds.

Vitzthum believes that the Hypoaspidinae are the most primitive, and from them have arisen on the one hand the Laelaptinae with enlarged plates, and on the other the ectoparasitic Liponyssinae and Dermanyssinae, through them the endoparasitic forms specialized in different directions. The sclerotization of the mites is, according to Vitzthum, a secondary development, the 'mentum' (*sensu* Berlese) is all that remains of the primary segmentation and represents the tritosternum, although Schulze (1935) believes that it is the deutosternum displaced backwards.

Vitzthum's ideas on the phylogeny of the subfamilies are not discussed here, but I accept his conclusion that the Parasitidae and Dermanyssidae (*sensu* Ewing) should be united. In this respect, I furthermore believe that among most acarologists there is a tendency to create too many small families compared, for instance, with the insects. This tendency only renders practical systematic work more difficult. The same applies to the genera. Radford, in his check-list of parasitic mites (1943),

separates, for instance, *Androlaelaps* and *Haemolaelaps* as good genera. Studying the parasitic mites of African rodents, I have found that there are all transitions from typical *Androlaelaps* with the second pair of legs strongly enlarged and calcarated (*zuluensis* n.sp.) to those of which the legs only bear a small tooth (*theseus* n.sp.). *Haemolaelaps capensis* Hirst, on the other hand, has the second pair of legs also distinctly enlarged and armed with strong spines, but a typical spur is missing. The differences between the weakly armed *Androlaelaps* and the strongly spined *Haemolaelaps* are so slight that their separation is sometimes a matter of great difficulty.

In the above-mentioned work, Vitzthum degraded the former genera *Haemolaelaps* Berlese, 1910 and *Androlaelaps* Berlese, 1903 (Berlese also described them as subgenera) to subgenera of *Hypoaspis* which is the type of this new subfamily Hypoaspidinae. The outstanding feature of the Hypoaspidinae is the tongue-shaped genital plate provided with only one pair of genital hairs. Many or most of the genera belonging to this subfamily contain free-living forms. The parasitic ones on vertebrates belong to *Hypoaspis* Canestrini, 1885, *Cyclolaelaps* Ewing, 1933, *Bolivilaelaps* Fonseca, 1940, *Mungosicola* Radford, 1942 and *Ugandolaelaps* Radford, 1942. Vitzthum has transferred the genera *Neoparalaelaps* Fonseca, 1937, and *Gigantolaelaps* Fonseca, 1939, to his subfamily of Laelaptinae. Because of the tongue-formed genital plate, I think they are better placed among the Hypoaspidinae. There are still some other groups, at present kept as genera, namely *Atricholaelaps* Ewing, 1929, *Eubrachylaelaps* Ewing, 1929, *Cavilaelaps* Fonseca, 1937 and *Ischnolaelaps* Fonseca, 1937. Only *Ischnolaelaps*, originally described from South America, contains Ethiopian species, according to Radford (1939). I am sure that this genus is a synonym of *Haemolaelaps*, taking into consideration the American as well as the Ethiopian species. The other three genera have been described from North and South America. I believe that they probably also belong to *Hypoaspis* and form at most subgenera of it.

The above-mentioned genera of Hypoaspidinae parasitic on vertebrates may be separated, in the female sex, as follows:

1. (12) Genital plate with one pair of spines or, when it is strongly reduced, these spines are placed near it. Hypoaspidinae **2**
2. (3) Dorsal plate divided, so that two shields are to be seen. Genital plate very strongly reduced, the pair of genital hairs standing behind it. Between coxae III and IV a pair of large stigmal plates, but no peritreme. Africa *Mungosicola* Radford, 1942
3. (2) Dorsal plate not divided. Genital plate mostly not reduced, tongue-shaped or enlarged and extending backwards; but with the usual pair of genital hairs at the base **4**
4. (11) Genital plate and anal plate not fused **5**
5. (10) Some of the coxae armed with toothlike spines **6**
6. (7) Spines on coxae I and II bifid. South America. *Neoparalaelaps* Fonseca, 1935
7. (6) Spines of coxae not bifid **8**
8. (9) Larger species, about 1½–2 mm. One of the posterior seta of coxa II always much larger than the other, generally extremely long. Body more or less oval. South America *Gigantolaelaps* Fonseca, 1939
9. (8) Smaller species, not reaching 1 mm. One of the posterior seta of coxa II not strikingly longer than the other. Body almost circular. North America *Cyclolaelaps* Ewing, 1933
10. (5) Coxae not armed with toothlike spines, they are always hair-like. Worldwide *Hypoaspis* Canestrini, 1885

    Two Ethiopian blood-sucking subgenera:

    (*a*) Second pair of legs more or less enlarged, femora calcarate. *Androlaelaps* Berlese, 1903

    (*b*) Second pair of legs also slightly enlarged, but femora not calcarate, with hairs or spines only. *Haemolaelaps* Berlese, 1910*
11. (4) Genital plate extended backwards and fused to the anal plate, thus forming a genito-ventral plate as in the Laelaptinae, but provided with only one pair of setae at the base. Africa *Ugandolaelaps* Radford, 1942
12. (1) Genital plate more or less enlarged and furnished with more than one pair of hairs thus forming a genito-ventral plate. Laelaptinae

* According to Berlese, the characteristic feature of *Haemolaelaps* is a very long seta on the fixed digit of the chelicerae. This seta, however, varies very much in the different species described up to date. The subgeneric division of the genus *Hypoaspis* is very unsatisfactory and confused. A revision must be undertaken with consideration also of the non-parasitic forms.

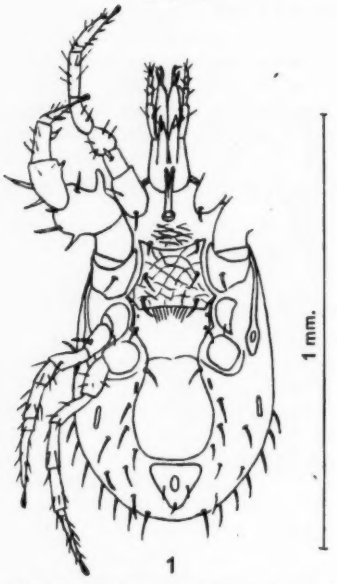
So far the South African ectoparasitic mites from rodents studied by me all belong to the two genera *Hypoaspis* and *Laelaps*. Among *Laelaps* there were no new species, and I have come across only one known *Hypoaspis*: namely *H.* (*Haemolaelaps*) *capensis* (Hirst). However, three new species belonging to the subgenus *Androlaelaps* were found and are described in this paper. Only one Ethiopian species of *Androlaelaps* has hitherto been described, namely *marshalli* Berlese, 1911, from British Somaliland. The description is very short, incomplete and without any drawings. The recognition of this species must therefore remain in doubt. So far I have not been able to trace the type material.

DESCRIPTION OF NEW SPECIES

The types of the new species described below are in the collection of the South African Institute for Medical Research, Johannesburg.

Hypoaspis (Androlaelaps) zuluensis n.sp.

(Fig. 1)



1

Fig. 1. Female venter of *Hypoaspis* (*Androlaelaps*) *zuluensis* n.sp.

FEMALE. The idiosoma of the single known specimen is 0·787 mm. long and 0·504 mm. broad (index: 1·56)*. Shape oval, without shoulders. Colour light brown.

Dorsum completely covered by the dorsal shield which is slightly reticulated. Hairs few, of moderate length and stoutness, tips a little recurved.

* Index = greatest length : greatest width.

The venter shows the sternal plate, which is widened posteriorly, to be broader than long (12:7); anterior margin slightly concave, posterior one almost straight. Reticulation distinct, asymmetrical. There is also a reticulated area in front of the sternal plate, but no presternal plate is developed. Sternal hairs short, the foremost pair placed on the edge of the plate. Pores indistinct.

Metasternal hairs level with posterior edge of coxa III flanked laterally by small plates. Near the middle of coxa III, the usual third pair of pores is to be seen.

Genital plate extending far backwards and separated only by a small space from the anal plate. It is tongue-shaped and widened at the middle. The pair of genital hairs are placed on the edge of the plate level with coxa IV. Flanking the genital plate there is one pair of very small spine-like plates, the metapodial plates behind coxa IV are oblong. Anal plate triangular, with three hairs.

Uncovered area of venter bearing eight pairs of hairs, three of which flank the genital plate. Stigmal pore between coxae III and IV, peritreme reaching forwards beyond coxa I.

Second pair of legs strikingly enlarged and armed with strong spines, femur bears on the inner side a large spur, genu a smaller one. The following two segments show on the inner side a lancet-shaped hair each. Coxae of legs I–III with only one hair, no tooth-like spines. Coxa IV bare.

Chelicerae with a long seta on the fixed digit, the movable one with two small teeth.

MALE and NYMPHAL STAGES unknown.

LOCALITY and HOST. Abercorn pont, Zululand, 12 May 1947, 1 ♀ from *Aethiomys namaquensis* (A. Smith).

Hypoaspis (Androlaelaps) africanus n.sp.

(Fig. 2)

FEMALE. The measurements of idiosoma vary from 0·923 × 0·650 mm. to 1·026 × 0·752 mm., index of the different specimens from 1·3 to 1·4. Shape short-oval, more compact than in *zuluensis*, shoulders not developed. Colour light brown.

The dorsal shield does not completely cover the dorsum, a narrow exposed border is visible posteriorly. Dorsal hairs few, of moderate length and stoutness, tips a little recurved.

Sternal plate (Fig. 2) nearly twice as broad as long, anterior and posterior margins concave. Reticulated area distinct bearing three pairs of strong hairs and two pairs of slit-shaped pores. The anterior pair of sternal hairs is placed on the edge of the plate. As in *zuluensis*, there is also a small reticulated area in front of the sternal plate.

Metasternal hairs nearly level with the posterior edge of coxa III and flanked laterally by small plates.

Near the middle of coxa III the third pair of pores is to be seen.

Genital plate not extending so far backwards as in *zuluensis*, the space between it and the anal plate much wider. Shape of genital plate also tongue-shaped, but more compact. The pair of genital hairs are placed on the edge of the plate. To the left and to the right of each of the first pair of hairs beside the genital plate, a very small vestigial plate is to be seen, the metapodial plates are well developed. Anal plate triangular, with three hairs.

Uncovered area of venter more densely haired than in *zuluensis*, but the three pairs of hairs flanking the genital plate arise from the same spots. Stigmal pore between coxae III and IV, peritreme reaching coxa I.

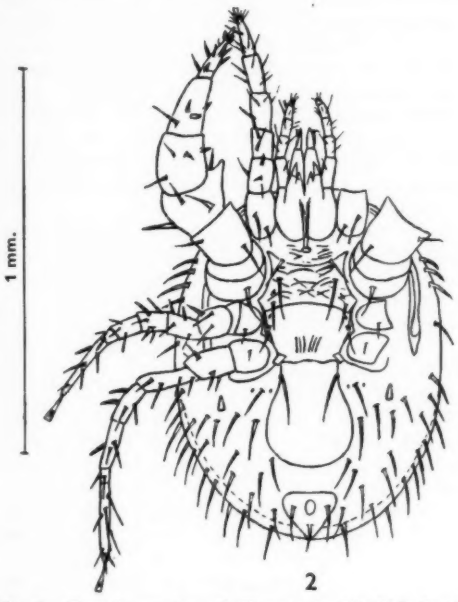


Fig. 2. Female venter of *Hypoaspis* (*Androlaelaps*) *africanus* n.sp.

Second pair of legs strongly enlarged, spur of femur very distinct, but in comparison to *zuluensis* a little smaller. All segments of this pair of legs beset with stout spines, but typical lancet-shaped ones as in *zuluensis* absent. Coxa I with two hairs, the others with one each.

Chelicerae with a strong seta on the fixed digit, the movable arm with two teeth.

MALE and NYMPHAL STAGES unknown.

LOCALITIES and HOSTS. Abercorn pont, Zululand, 12 May 1947, 2 ♀♀ (one the Holotype) from *Saccostomus* sp.; Kachikau, Bechuanaland, 23 December 1944, 5 ♀♀ from *Thallomys damarensis* (de Winton); Sebena, Bechuanaland, 26 November 1944, 1 ♀ from *Tatera lobengulae* (de Winton) and 1 ♀ from *Saccostomus campestris* Peters; Sehitwa, Bechuanaland, 9 December 1944, 5 ♀♀ from *Desmodillus auricularis* (A. Smith).

Hypoaspis (Androlaelaps) theseus n.sp.
(Figs. 3 and 4)

FEMALE. The measurements of idiosoma vary from 1·385 × 1·053 mm. to 1·607 × 1·219 mm., index from 1·3 to 1·4. Shape short-oval, as compact as in *africanus*. Colour light brown.

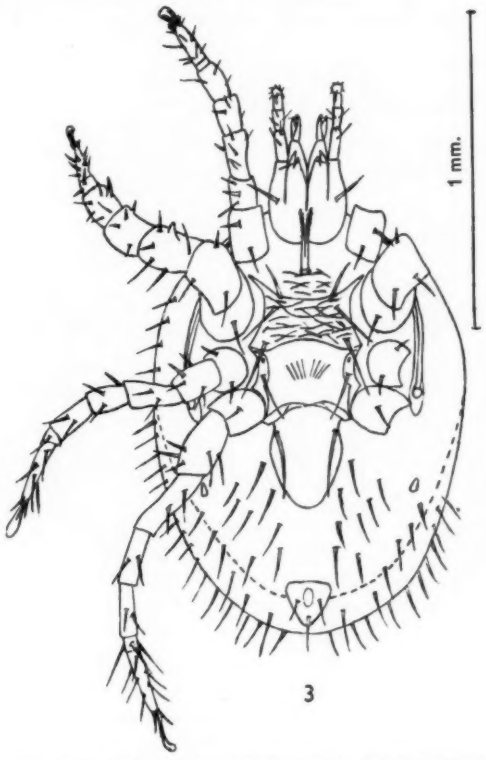


Fig. 3. Female venter of *Hypoaspis* (*Androlaelaps*) *theseus* n.sp.

The dorsal shield does not completely cover the dorsum, the exposed border mostly wider than in the preceding species. Dorsal hairs few, of moderate length and stoutness, tips a little recurved.

Sternal plate nearly twice as broad as long, anterior and posterior margins deeply concave. Reticulated areas, hairs and pores as in *africanus*.

Metasternal hairs placed on the plates and these are a little wider than in the foregoing species. Third pair of pores near the middle of coxa III distinct.

Genital plate smaller and shorter than in *africanus*, the greatest width lies before the middle. The shape however, varies a little. There are specimens of which the plate shows no, or nearly no, widening. The plate may also be slightly asymmetrical. Genital hairs placed on the edge of the plate. No vestigial plates in the vicinity of the genital plate, but the metapodial plates are distinct and as well developed as in *africanus*. Anal plate triangular, with three hairs.

Uncovered area of venter as densely haired as in *africanus*, near the genital plate are the usual three pairs of hairs. Stigmal pore between coxae III and IV, peritreme reaching coxa I.

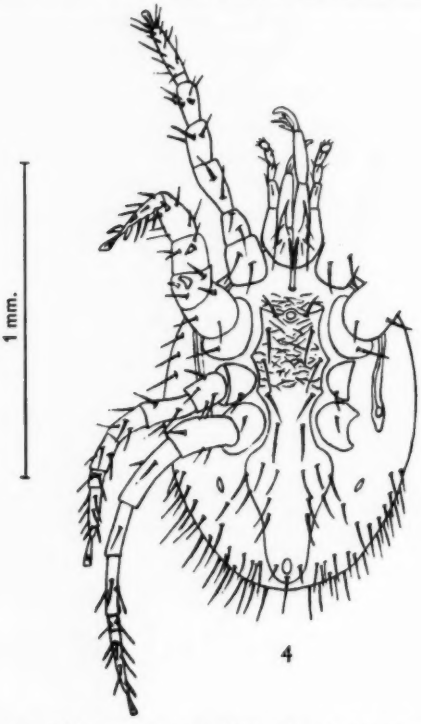


Fig. 4. Male venter of *Hypoaspis* (*Androlaelaps*) *theseus* n.sp.

Second pair of legs not so strongly enlarged as in *africanus* and *zuluensis*, spur of females smaller. It may be difficult to see the spur in poor preparations. It should be noted that this spur is a typical one and not a spine which is always placed in a pit. Therefore, *theseus* belongs to *Androlaelaps* and not to *Haemolaelaps*. The other armature consists of spines which are sometimes very short and stout. Coxa I with two hairs, the others with one each.

Digits of chelicerae with distinct teeth and one long seta on the movable member.

MALE. Idiosoma varies from 1·080 × 0·776 mm. to 1·247 × 0·914 mm., index being 1·3 to 1·4. Egg-shaped, a little pointed posteriorly. Colour as in female.

Dorsal shield entirely covers the dorsum, chaetotaxy as in the female.

Venter shows a shield consisting of the united sternal, metasternal, genital and anal plates. In front of it, a reticulated area is to be seen, but a separate presternal plate has not been developed. Anterior margin of ventral shield concave, with the genital orifice in the middle. Lateral margins incurved at the coxae, widened behind coxa IV, then gradually reduced to the anal opening. First pair of sternal spines placed on the edge of the anterior margin to the left and right of the genital pore. Second pair of spines level with middle of coxa II, third pair level with its posterior edge. Metasternal pair of spines slightly posterior to middle of coxa III. Genital pair of spines placed about the middle of coxa IV. Posterior to coxa IV there are usually four pairs of spines, not including the three anal spines, but sometimes there is one doubled.

Uncovered area densely beset with spines, metapodial plates distinct. Stigmal pore between coxae III and I, peritreme reaching coxa I.

Chaetotaxy of legs as in female, but spur of femur II mostly more distinct.

Chelicerae with lengthened mandibles transformed for copulatory purposes.

NYMPHAL stages unknown.

LOCALITIES and HOSTS. Wakkerstroom, Transvaal, 5 May 1947, 2 ♂♂, 10 ♀♀ from *Mastomys coucha* (A. Smith), among these Holotype ♀ and Allotype ♂, and 5 ♂♂, 8 ♀♀ from *Tatera brantsii* (A. Smith); 20 miles on Piet Retief–Paulpietersburg Road, Natal, 6 May 1947, 2 ♀♀ from *Tatera brantsii* (A. Smith); Ndumu, Zululand, 13 May 1947, 2 ♂♂, 7 ♀♀ from *Tatera lobengulae* (de Winton); Sebena, Bechuanaland, 26 November 1944, 4 ♀♀ from *Tatera lobengulae* (de Winton); Kanyo, Bechuanaland, 29 November 1944, 5 ♂♂, 3 ♀♀ from *Tatera brantsii* (A. Smith); Sebena, Bechuanaland, 26 November 1944, 1 ♂, 2 ♀♀ from *Aethiomys chrysopilus* (de Winton).

I wish to express my hearty thanks to Dr De Meillon, Entomologist at the South African Institute for Medical Research, Johannesburg, for his help and advice in my studies. Moreover, I am indebted to Mr Davis of the Plague Research Laboratory, Union Health Department, for having placed at my disposal the collection of rodent-infesting mites.

REFERENCES

BERLESE, A. (1903). Diagnosi di alcune nuove specie di Acari italiani mirmecofili e liberi. *Zool. Anz.* **27**, 12.

BERLESE, A. (1903). Illustrazione iconografica degli acari mirmecofili. *Redia*, **1**, 299.

BERLESE, A. (1911). Acarorum species novae quindecim. *Redia*, **7**, 429.

EWING, H. E. (1929). *A Manual of External Parasites.* London: Baillière, Tindall and Cox.

EWING, H. E. (1933). New genera and species of parasitic mites of the super-family Parasitoidea. *Proc. U.S. Nat. Mus.* **82**, 1.

FONSECA, F. DA (1937*a*). New genera and species of Acari 'Laelaptidae' from Brazilian rodents. *XII Int. Congr. Zool., Lisbon*, 1935, p. 1597.

FONSECA, F. DA (1937*b*). Notas de Acareologia. XXIV. A. Representante brasileiro do genero *Dermanyssus* Duges, 1834 (Acari dermanyssidae). B. Nota sobre o nome generico *Paralaelaps*. *Mem. Inst. Butantan.* **10**, 51.

FONSECA, F. DA (1939). Notas de Acareologia. XXV. Os Laelaptidae gigantes, parasitas de roedores sul-americanos; genero e especies novos (Acari). *Mem. Inst. Butantan.* **12**, 7.

FONSECA, F. DA (1940). Notas de Acareologia. XXXI. *Bolivilaelaps tricholabiatus*, gen.n., sp.n. (Acari, Laelaptidae). *Mem. Inst. Butantan.* **14**, 59.

RADFORD, C. D. (1939). Notes on some new species of parasitic mites. *Parasitology*, **31**, 243.

RADFORD, C. D. (1942*a*). New ectoparasitic mites (Acarina) from Uganda. *Parasitology*, **34**, 185.

RADFORD, C. D. (1942*b*). New parasitic mites (Acarina). *Parasitology*, **34**, 295.

RADFORD, C. D. (1943). Genera and species of parasitic mites (Acarina). *Parasitology*, **35**, 58.

SCHULZE, P. (1935). Zur vergleichenden Anatomie der Zecken. *Z. Morph. Ökol. Tiere*, **30**, 1.

VITZTHUM, H. GRAF (1943). Acarina. In Bronn, *Klassen u. Ordnungen d. Tierreiches*, **5**, IV. Abt., 5. Buch. Leipzig: Becker and Erler.

(*MS. received for publication* 1. III. 1949.—Ed.)

ABNORMAL MATERIAL BEARING ON THE DEVELOPMENT OF THE TERMINALIA OF MALE CALYPTERATE CYCLORRHAPHOUS DIPTERA

By H. T. THOMAS, *Department of Zoology, University of Cambridge*

(With Plate VI and 7 Figures in the Text)

CONTENTS

	PAGE
I. Introduction	304
II. Description of the two abnormal specimens	
(1) Specimen I	304
(2) Specimen II	308
III. Discussion	
(1) Crampton's series of male terminalia	311
(2) Hardy's views on the development of the circumverted terminalia	312
(3) A comparison of Specimens I and II with Crampton's series and their interpretation as developmental stages	313
(4) A note on the internal anatomy of Specimens I and II	314
(5) Further interesting points in the terminalia of normal and the abnormal specimens	314
IV. Summary	315
References	315

I. INTRODUCTION

This paper gives an account of the terminalia of two abnormal male Diptera belonging to two species of the genus *Sarcophaga* Meigen. These specimens appear to throw light on the nature of the inverted, curvilinear and circumverted types of terminalia, and on the segmental numbering of the abdominal sclerites, in the males of higher Diptera. The material seems to support Crampton's views (1944) on these problems, and suggests what may have been the course of evolution of the terminalia of male calypterate cyclorrhaphous Diptera.

Briefly, the facts are as follows:

(1) Specimen I, *S. similis* Meade, exhibits both a 90° curvilinear displacement and a 90° 'circumversion' of the terminalia.

(2) Specimen II, *S. hirtipes* Wiedemann var. *orchidea* Böttcher, exhibits a simple 180° inversion of the terminalia.*

(3) In Specimen II, the pregenital plate† is represented by a sclerite which appears to be part of the normal pregenital plate, that is, of synsternite 7–8. It is suggested that this sclerite is sternite 7.

As these specimens are of the genus *Sarcophaga* reference will mainly be made to the normal anatomy of species of that genus,* but the conclusions drawn from this study apply to other allied genera as well.

There is some confusion in the literature in the descriptions of the parts of the terminalia of male Cyclorrhapha because there is as yet no generally accepted conventional position in which the terminalia are described. To clarify the position, the normal terminalia will be described when: (1) the segments are fully protracted so that they follow each other as nearly in a horizontal sequence as possible and so as to direct the anal opening backwards; (2) the aedeagus is pushed as far forwards as possible—it generally comes to rest at an angle of about 45° to the horizontal. The condition of the terminalia is now in the usual protracted position of mounted *Sarcophaga* specimens. With the terminalia in this position, Hardy's definitions (1944) are made precise.

Crampton's names (1944) for the various parts of the terminalia will be used throughout the paper.

II. DESCRIPTION OF THE TWO ABNORMAL SPECIMENS

(1) *Specimen I*

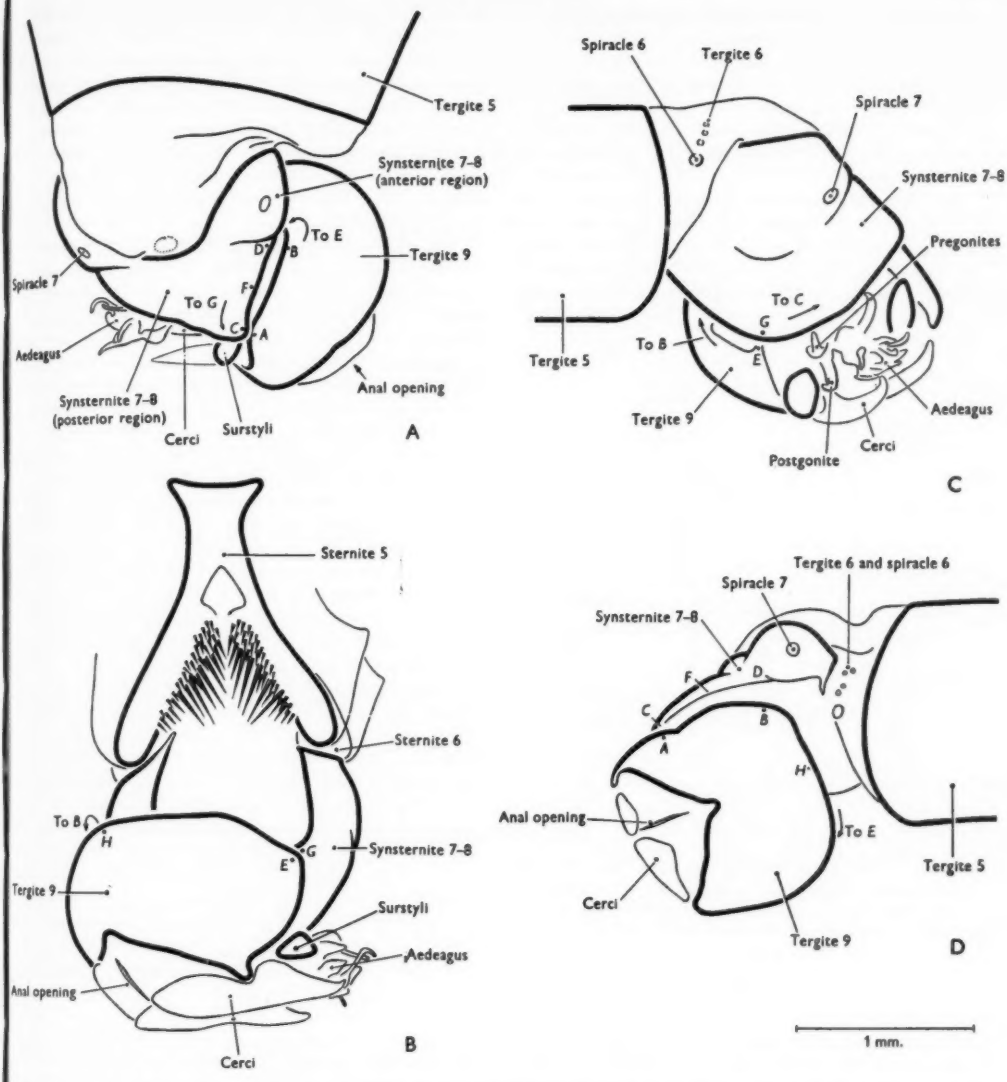
Sarcophaga similis Meade

The systematic description of this specimen conforms to that of *Sarcophaga similis* Meade (see description in Senior White, Aubertin & Smart, 1940), but the terminalia show certain abnormalities.

* The angles are measured with reference to the position of the parts in a normal specimen.

† In the descriptions of normal specimens, the terms 'pregenital plate' and 'synsternite 7–8' (i.e. according to Crampton, the product of the fusion of sternites 7 and 8) are synonymous. Where doubt exists as to the composition of this sclerite, as in Specimen II, the more non-committal term 'pregenital plate' will be used.

* For a general description of the normal anatomy of the terminalia of *Sarcophaga*, see Patton (1934). The labelling of the sclerites does not conform to those used in this paper.

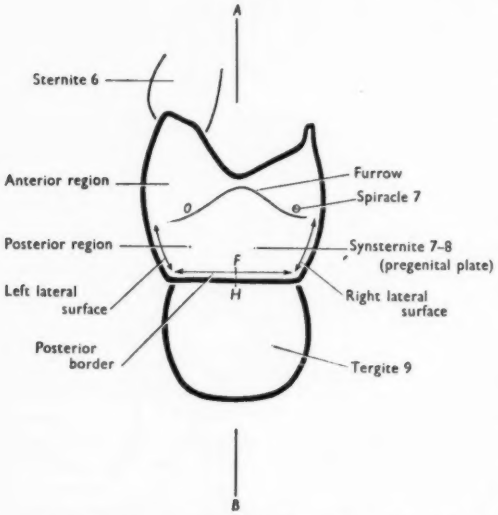


Text-fig. 1. *Specimen I.* A, dorsal aspect of the terminalia. Dotted circle marks the position of the unusual ingrowth of intersegmental membrane. B, ventral aspect of the terminalia. C, left lateral aspect of the terminalia. Broken circles indicate the positions of tergite 6 and spiracle 6. D, right lateral aspect of the terminalia.

Description of the terminalia. Tergite and *sternite* 5 are normal (Text-fig. 1 A, B). *Tergite* 6 (Text-fig. 1 C, D) is normal; its position is indicated by a short, single line of bristles (5–6 in number) on each side of the intersegmental membrane between tergite 5 and synsternite 7–8. The membrane around the bristles is unsclerotized. *Spiracles* 6 (Text-fig. 1 C, D) are in their normal position in the membrane at the lower end of the line of bristles representing tergite 6. (In Text-fig. 1 C, tergite 6 and its spiracle are hidden by a fold of membrane; their positions are indicated by the line of broken circles.) The relations of *sternite*

6 (Text-fig. 1B) are normal; it articulates with the mid-dorsal surface of sternite 5 and the left anterior border of synsternite 7–8, and lies on the left side of the terminalia. (Text-fig. 1B may give the impression that sternite 6 articulates with the left inferior extremity of the anterior border of synsternite 7–8: this is not so. The articulation occurs some distance above the inferior extremity of the synsternite.)

The membrane between tergite 5 and synsternite 7–8, in which lies tergite 6, is slightly twisted from right to left (Text-fig. 1A). Posteriorly, in a dorsal position and quite close to the synsternite, an unusual ingrowth of the membrane has occurred. It is pig-



Text-fig. 2. Dorsal aspect of a normal synsternite 7–3 of *Sarcophaga*, showing its relations and characteristics. *AB*, antero-posterior axis of the abdomen. *F*, mid-dorsal point of the posterior border of synsternite 7–8. *H*, mid-dorsal point of the anterior border of tergite 9.

mented and very small. This abnormality is probably without significance (see Text-fig. 1A, dotted circle).

In order to make clear the relation between synsternite 7–8 of the abnormal specimens and that of a normal specimen, it will be necessary to give a detailed description of the normal condition.

In the normal terminalia synsternite 7–8 (Text-figs. 2, 6B, D) is a dome-shaped sclerite, dorsal in position, and roughly bilaterally symmetrical about the longitudinal axis of the abdomen. It is usually as long as it is wide, and its lateral margins reach to a little more than half-way down the sides of the terminalia; the membrane which stretches between the two lower borders forms a groove (part of the cubiculum) which contains the aedeagus, cerci, etc., when the terminalia are retracted. The synsternite is held in position by two rigid structures: anteriorly (and to a limited extent) by sternite 6, and posteriorly by tergite 9. The dorsal surface of the synsternite is marked by a furrow, shaped like a widely opened V with apex pointing anteriorly, which is distinct dorsally, but gradually fades away at the sides of the sclerite. The furrow divides the sclerite into approximately equal anterior and posterior regions.

The anterior region of synsternite 7–8 is roughly hour-glass-shaped in outline as seen from above (Text-figs. 2, 6D). Its two halves are raised in shallow relief. The anterior border is deeply notched dorsally, and there is a fairly conspicuous process (? tergite 7) at the right infero-lateral corner (Text-fig. 6B). A small part of the anterior border articulates with sternite 6; this articulation occurs some distance above the left inferior extremity of the border.

The posterior region of synsternite 7–8 is roughly rectangular in outline when viewed from above; its sides are slightly curved (Text-figs. 2, 6D). The two lateral surfaces, of equal length, end inferiorly in the inferior borders of the synsternite. The posterior border of the part articulates along its whole length with tergite 9; the inferior angles are slightly knobbed (Text-fig. 6B) and provide what appear to be the strongest points of articulation with the tergite.

Both regions of synsternite 7–8 are hairy, and along the inferior borders the hairs are often particularly long. In some species a row of strong bristles (the so-called 'marginals' in taxonomy) is to be found on the hind border of the posterior region. The anterior region is 'shining'—never coated with 'pollen'; the posterior region may or may not be so coated. In taxonomical work when 'genital segment 1' is described as pollinose, it is only the posterior region which is affected.

The pair of spiracles (spiracles 7) may be situated in the anterior region, in the posterior region, or in the furrow separating the two regions of the synsternite. Their position does not seem to be constant for a given species.

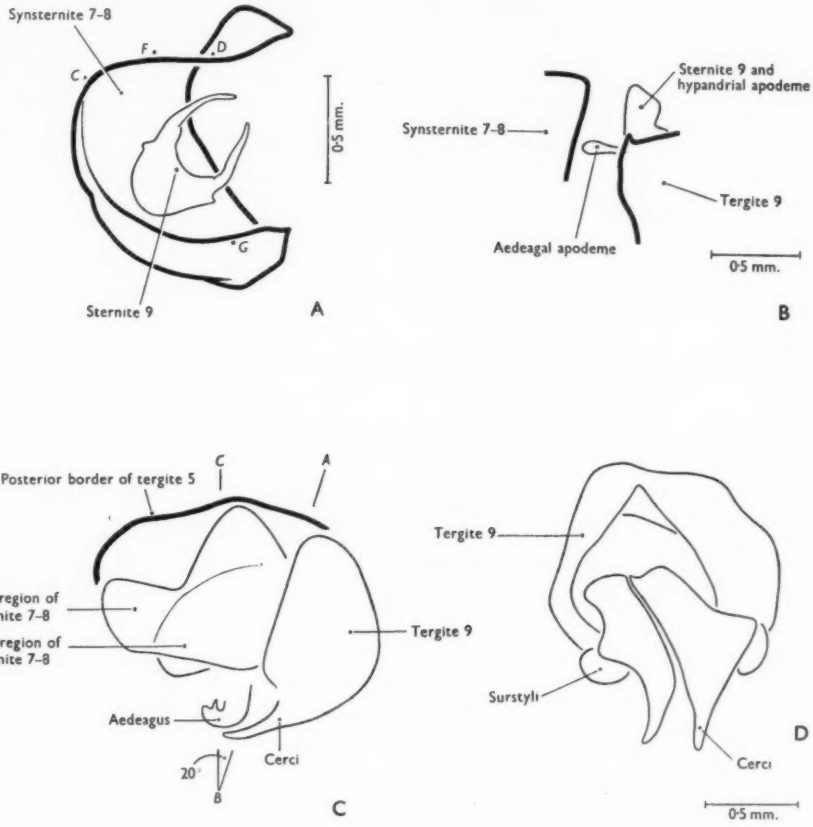
Synsternite 7–8 of Specimen 1 (Text-fig. 1A) is unusual and is slightly displaced to the left of the terminalia (Text-fig. 3C; Pl. VI, fig. 1). It is a single sclerite but is divided by a furrow into anterior and posterior regions. The anterior region is apparently normal: it is slightly inflated, the anterior border is bifid dorsally, it carries a right infero-lateral process (Text-fig. 1D), it articulates with sternite 6 and bears the last pair of spiracles (*spiracles* 7). Its surface is also shining (i.e. it is not covered with pollen).

The posterior region of synsternite 7–8 is asymmetrically developed. Seen from above (Text-fig. 1A), it is triangular in outline, with an exposed posterior surface and a right lateral border (*CD*,

Text-fig. 1A) articulating with tergite 9. The left lateral aspect of this region (Text-fig. 1C) is more or less normal in appearance, except that the inferior border descends lower than usual, and the posterior border is rather lost sight of as the lateral surface sweeps round to the right. With tergite 9 removed, the synsternite appears as a hollow sclerite when viewed from the right side (Text-fig. 3A) or from below. The external surface is pollenose.

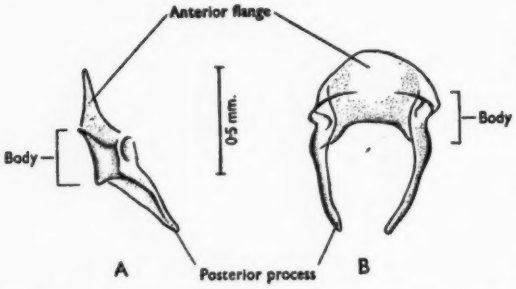
The peculiar construction of the synsternite results in the first abnormal displacement of the terminalia —a 90° turn to the right in the horizontal plane.

We must now consider those segments which follow synsternite 7–8. It may be said at once that tergite 9 and its associated structures (the whole complex sometimes termed the hypopygium) are normal, both in shape and in their relations one with another; in this specimen, as in a normal specimen, the infero-lateral borders of tergite 9 articulate with the following structures, from before backwards: (1) sternite 9, (2) the surstyli and (3) the cerci. Sternite 9, in turn, articulates with the following structures, again from before backwards: (1) tergite 9, (2) the pregonites, (3) the postgonites and (4) the aedeagus. In Specimen I, tergite 9 and the anal cerci appear slightly twisted, however (Text-fig. 3D), but this does not disturb the relations of the various parts just described. The aedeagus is normal and is typical



Text-fig. 3. *Specimen I.* A, a right lateral view of synsternite 7–8. Sternite 9 has been restored to the position which it occupied before dissection. B, a lateral view of synsternite 7–8 and tergite 9, slightly separated during dissection, showing the relation of the synsternite and sternite 9. C, a view of the intact terminalia seen from behind: a tracing of Pl. VI, fig. 1. *AB* indicates the plane of articulation of synsternite 7–8 and tergite 9; the plane is set at an angle of about 20° from the vertical (*CB*). D, posterior aspect of tergite 9, the cerci and surstyli, showing their twisted appearance.

of the species *S. similis*. The relations of the articular surfaces of synsternite 7–8 and tergite 9 are abnormal however: tergite 9 fits on to the right lateral border of synsternite 7–8 in such a way that the aedeagus and cerci are directed to the left, so that the genital opening points to the left and the anal opening to the right (Text-fig. 1). It will be seen from Text-fig. 1 that only restricted regions of the articular border of tergite 9 articulate with the right lateral border of synsternite 7–8, viz. section *AB* (Text-fig. 1 A, D) and point *E* (Text-fig. 1 B, C) of the former with section *CD* (Text-fig. 1 A, D) and point *G* (Text-fig. 1 B, C) of the latter. A large portion of the border of tergite 9, namely, from *B* to *E* (Text-fig. 1 A, B, C, D), is free. It seems evident that tergite 9 has rotated upon the end of the synsternite away from the normal position (see under Discussion); this is the second important anomaly of the terminalia.



Text-fig. 4. *Specimen I*. A, left lateral aspect of sternite 9. B, upper aspect of sternite 9. The anterior flange, body and posterior processes are equivalent to the hypandrial apodeme, sternite 9 and posterior arms respectively of Crampton.

In normal specimens there is only one region of contact between synsternite 7–8 and the hypopygium, that is, along the posterior border of the synsternite and the anterior border of tergite 9 (Text-fig. 2). In this specimen, however, another point of contact occurs between the synsternite and hypopygium: the hypandrial apodeme of sternite 9 ('anterior flange', Text-fig. 4) comes into close contact with one of the borders of synsternite 7–8 (Text-fig. 3 A). Sternite 9 (Text-fig. 4) is, however, normal. The relations and attachments of sternite 9 have been dealt with already (see above, and also under Discussion). The sclerite of a normal specimen (Text-fig. 4) consists of a middle part or body (Crampton's sternite 9), an anterior flange (Crampton's hypandrial apodeme) and two posterior processes (Crampton's posterior arms). Other prominences (found, for example, in *S. hirtipes* var. *orchidea* (Text-fig. 6 F) and flanges (found, for example, in certain species of *Calliphora*) may also be present. The body is an external structure which forms the posterior boundary of the cubiculum; the anterior flange and the two posterior processes, on the other hand, are internal structures. In this specimen the anterior flange (the hypandrial apodeme) of this sclerite makes contact with synsternite 7–8.

In spite of the apparent rotation of tergite 9, the intersegmental membrane between synsternite 7–8 and tergite 9 does not appear to be twisted; the two sclerites are, in fact, too close together for any such twist to be apparent.

Internal anatomy. While the parts of the terminalia were being disarticulated it appeared that the genital duct did not loop over the alimentary canal (as in normal specimens), but as both gut and duct were so fragile and transparent this cannot be stated with certainty. When the dissection was completed the preparation resembled the dissection of Specimen II shown in Text-fig. 7 C.

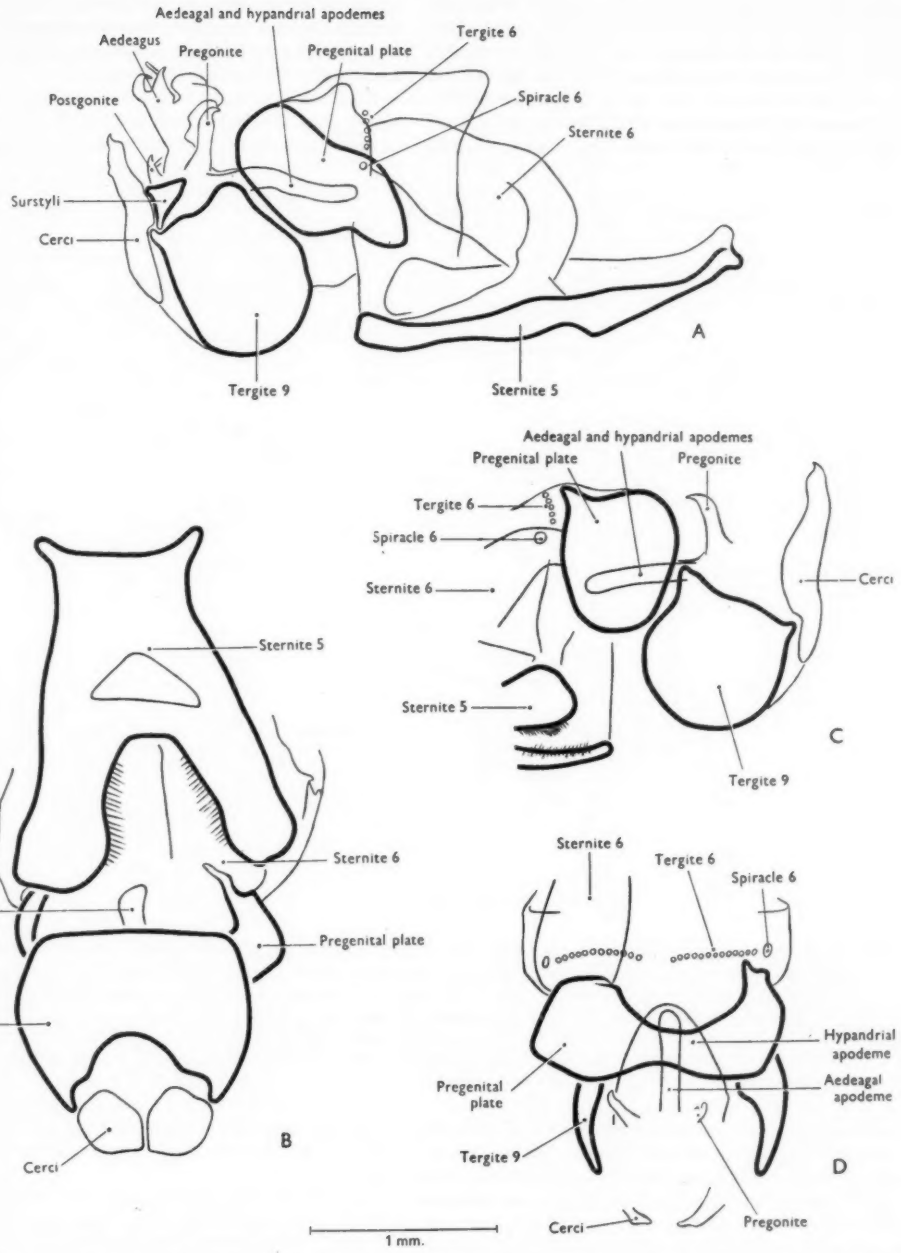
This specimen came from a batch of individuals reared in the laboratory from a single female. The other males and females (about ten in all) were normal. It is important to note that this individual was viable.

(2) *Specimen II*

Sarcophaga hirtipes Wiedemann var. **orchidea** Böttcher

The taxonomic description of this specimen agrees with that of *S. hirtipes* var. *orchidea* (see description in Senior White *et al.* 1940). The specimen is abnormal in that tergite 9 and its associated structures (the hypopygium) is rotated through 180° about the end of the pregenital plate away from the normal position. The bilateral symmetry of the whole abdomen is not disturbed, since there is no curvilinear development of the terminalia.

Description of relevant parts of the terminalia: (*a*) The *pregenital plate* ('synsternite 7–8') (Text-fig. 5). Compared with a normal synsternite of the same species, this sclerite is very short. On close inspection it is evident that the sclerite, rather than being malformed, is defective in that a part is lacking. It will be recalled (see description under Specimen I) that two regions can be distinguished in synsternite 7–8 of normal *Sarcophaga*: an anterior and a posterior region; the two are separated by a V-shaped furrow. In this specimen it is evident that only the anterior region is present, the posterior region is lacking. If this specimen be compared with a normal individual, e.g. in Text-fig. 6 A, B, C, D, it will be seen that the outline of drawings of Fig. 6 A and C (the abnormal pregenital plate) can be superimposed on that of the homologous part (i.e. the anterior region) of Text-fig. 6 B and D (a normal synsternite of the same species) respectively. Note that the anterior region seems to have been separated



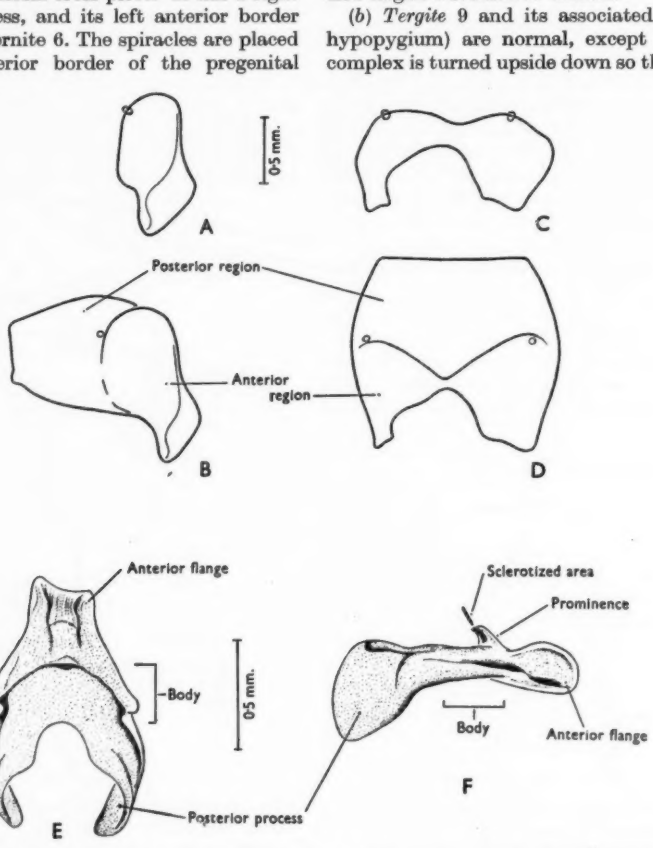
Text-fig. 5. *Specimen II*. A, right lateral aspect of the terminalia. B, ventral aspect of the terminalia. C, left lateral aspect of the terminalia. Parts of the genital apparatus (e.g. the aedeagus, etc.) have been omitted. D, dorsal aspect of the terminalia. Parts of the genital apparatus have been omitted.

from the posterior region (absent) along a line of cleavage corresponding exactly to the V-shaped furrow on a normal synsternite. The pregenital plate of Specimen I consists then of two lateral rounded lobes joined by a dorsal cross-piece. It has a right infero-lateral process, and its left anterior border articulates with sternite 6. The spiracles are placed close to the posterior border of the pregenital plate, where the posterior border curls downwards to articulate with tergite 9 (Text-fig. 7A, B). Lastly, as with the anterior region of the pregenital plate in all *Sarcophaga*, this sclerite is lightly clothed with hairs, and is shining, not pollinose.

Because of the rotation of tergite 9 through 180°, articulation between the pregenital plate and tergite 9 is impossible in the dorsal region and can only occur between the lateral regions of the pregenital plate and tergite 9 (Text-fig. 5A, C). This means that the length of the line of contact between the two sclerites is considerably shortened. In spite of this shortening, however, the connexion between the pregenital plate and tergite 9 seemed to be firm.

(*b*) *Tergite* 9 and its associated structures (the hypopygium) are normal, except that the whole complex is turned upside down so that the aedeagus and cerci face upwards, while tergite 9 faces downwards; the anal opening, however, still points backwards.

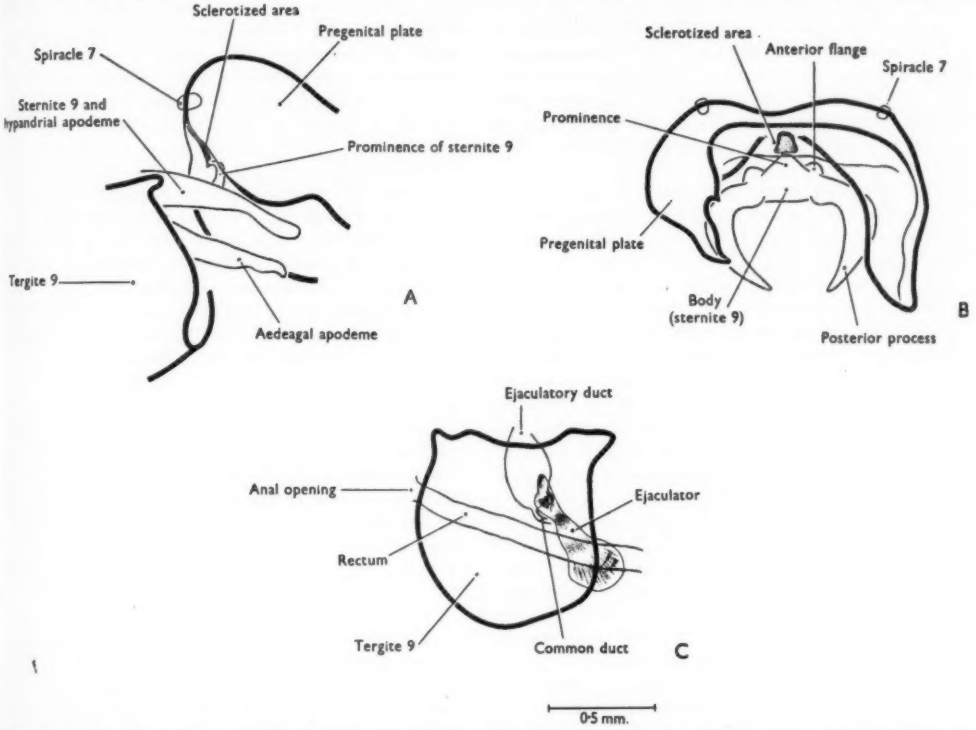
(*c*) *Sternite* 9 (Text-fig. 6E, F). This sclerite is normal. A prominence arises from the body of the sclerite, that is, from its middle region, and in a normal specimen this serves as the posterior median attachment of the membranous cubiculum. In this abnormal specimen, the prominence has rotated



Text-fig. 6. A, right lateral aspect of the pregenital plate of Specimen II. B, right lateral aspect of the pregenital plate (synsternite 7–8) of a normal individual of the same species (*S. hirtipes* var. *orchidea*). C, dorsal aspect of the pregenital plate of Specimen II. D, dorsal aspect of the pregenital plate (synsternite 7–8) of a normal individual of the same species. E, upper aspect of sternite 9 of Specimen II; in a normal specimen, this would be the lower aspect of the sclerite. F, right lateral aspect of sternite 9 of Specimen II, showing the prominence and its attachment to the small sclerotised area. The anterior flange, body and posterior processes are equivalent to the hypandrial apodeme, sternite 9 and posterior arms of Crampton.

through 180° away from the normal position and comes to lie close below the mid-dorsal posterior border of the pregenital plate (Text-fig. 7A, B). The membrane which stretches between the prominence and the posterior border of the pregenital plate appears as a very small, sclerotized and pigmented, median area (Text-fig. 7A, B).

Internal anatomy. As the dissection proceeded, it appeared that the genital duct did not coil over the alimentary canal. The final state of the dissection is shown in Text-fig. 7C.



Text-fig. 7. *Specimen II.* A, a lateral view of the pregenital plate and tergite 9, slightly separated. The dissection shows the relationship between sternite 9 and the pregenital plate. B, posterior view of the pregenital plate. The position of sternite 9 is indicated (it is greatly foreshortened from this view). C, tergite 9, the alimentary canal (rectum) and the genital duct (common and ejaculatory ducts) *in situ*. When the dissection had reached this stage in Specimens I and II only the short length of the common duct shown remained intact.

This specimen was caught in the field in a fly-trap baited with frog-meat—it was therefore viable in spite of its abnormality.

III. DISCUSSION

(1) *Crampton's series of male terminalia*

Crampton has made a comparative morphological study of the terminalia of male Cyclorrhapha. Starting with the simpler forms of Diptera he has shown how the various types of male terminalia in lower and higher forms can be arranged in a series displaying progressive modifications of an initial type found in the simpler forms. On the basis of this study he has endeavoured to homologize the various structures and has devised for these a system of nomenclature applicable throughout the Diptera. The two abnormal specimens of *Sarcophaga* described in this paper seem to have a bearing on the various stages in Crampton's series—indeed, they seem to reflect certain important steps in the series.

Crampton's series is a series of adult forms; but the occurrence of apparent stages in aberrant forms of a single genus that seem to correspond to certain members of the series suggests that some, at any rate, of the members of Crampton's series are also stages in the development of the higher Diptera. It is conceivable that development has been arrested prematurely in the abnormal specimens, and it is therefore suggested, on the evidence provided by these specimens, that the terminalia of higher

Diptera undergo torsion during pupal development through stages similar to those in Crampton's series of adult forms.

That torsion does occur during early pupal development has been shown by Schräder (1927) in *Calliphora*. Sir S. R. Christophers (personal communication, 1948), using *Sarcophaga* material, has also shown by serial sectioning of pupae that torsion of the terminalia takes place in the formation of the imago.

On the other hand, if these abnormal specimens represent arrested stages in development, one might expect that segment 6, and segment 9 and its associated structures, would also present some unusual anatomical features, suggesting that their development has been prematurely arrested; but, in fact, they are normal. It is only the pregenital plate which is abnormally developed in these specimens.

The following description of the various stages leading up to the normal condition of the terminalia of male Cyclorrhapha is based on that given by Crampton (1944).

(1) In the first stage, to be seen, for example, in the male mosquito immediately after emergence from the pupa, all the tergites and sternites of the abdomen succeed one another in a straight line; all tergites are dorsal and all sternites ventral. The anal opening (dorsal in position) and the genital opening (ventral in position) are directed backwards. Correlated with the positions of these openings the alimentary canal and genital duct are not crossed—the latter runs beneath and parallel to the former.

(2) Then follows a clockwise* rotation of the ninth segment through 180° about the longitudinal axis of the body, resulting in the inversion of the ninth segment, so that the genital opening now becomes dorsal in relation to the anal opening; both openings are directed backwards as before, but the apparent dorsal and ventral edges of the genital opening are, morphologically speaking, ventral and dorsal respectively. The bilateral symmetry of the terminalia is undisturbed as in stage (1). This inversion of segment 9 means that the genital duct crosses the alimentary canal in the plane defined by the antero-posterior and dorso-ventral axes; the crossing is evident when the specimen is viewed from the side. In the mosquito, stage (2) is reached several hours after emergence.

(3) There is next a forward-folding of the ninth segment against the right side of the terminalia, as in certain Syrphidae, e.g. in *Eristalis tenax* L. This is the curvilinear condition in which the inverted genital and anal openings project forwards instead of backwards; the true dorsal and ventral edges of the genital opening are still reversed as in stage (2). The terminalia has become asymmetrical about the longitudinal axis. The crossed condition of the alimentary canal and genital duct seen in stage (2) has not changed, but may be obscured as a result of the forward-folding of segment 9.

(4) The final condition is reached when segment 9 undergoes the circumversion movement which seems to consist in a complex twisting of the terminalia so that segment 9 (tergite 9) rotates still further about the end of segment 8 while sternites 6, 7 and 8 migrate around the left side of the terminalia to their final position in the normal adult, that is to say, sternite 6 comes to lie obliquely across the left side of the terminalia, while sternites 7 and 8 (which fuse to form synsternite 7–8) come to be dorsal in position. (The sternites have probably already begun to migrate in stage (3).) A superficial bilateral symmetry of the termination develops secondarily. From stage (3) to stage (4) there seems to be a further 180° torsion of tergite 9, so that in the final condition the genital duct makes a complete loop of 360° around the alimentary canal (the condition seen in normal adult Cyclorrhapha). The genital opening (the aedeagus) now points forwards and downwards, and the anal opening, backwards; the true ventral and dorsal edges of the aedeagus* are still facing dorsally and ventrally respectively as in the previous stage (the conventional position of the terminalia described in the Introduction is used).

(2) *Hardy's views on the development of the circumverted terminalia*

Hardy (1944) pointed out that 'authors had overlooked the fact that the aedeagus had remained inverted in an apparently erect hypopygium' and that therefore 'the idea that the hypopygium had turned through 360 degrees... may now be regarded as a misunderstanding'. By 'aedeagus inverted' he means that 'the true venter of the aedeagus lies in a plane nearer that of the anus than does its dorsum', and by 'erect hypopygium' he means that 'the anus lies in a plane above that of the aedeagus...' (see Introduction).

It is evident from the account of the development of the terminalia given here that the inverted condition of the aedeagus found in stage (3) retains its

* So far as is known, only clockwise rotation occurs in the terminalia of Cyclorrhapha. In mosquitoes, however, rotary inversion of the terminalia seems to take place in a clockwise or in an anti-clockwise direction, the *anti-clockwise* torsion, however, seems to be twice as frequent as the clockwise torsion. (Personal observation on *Deswoidya* Blanchard; number of specimens studied, 33.)

* It is presumed that the side of the aedeagus which carries the processes: hypophallus, paraphallus, etc., is the true ventral region of the aedeagus although it faces dorsally, and that the smooth opposite side is the true dorsal region of the aedeagus although it faces ventrally. Normally, therefore, the aedeagus is inverted.

inverted condition after circumversion occurs (stage (4)). Hardy's point about the presence of the inverted aedeagus in the adult terminalia (with its erect hypopygium) is not incompatible, therefore, with the occurrence of a 360° revolution of tergite 9 (or the hypopygium).

In outlining the phylogenetic development of the circumverted terminalia, Hardy proceeds through stages (1), (2) and (3) as described here, but ends his description with the aedeagus in the curvilinear stage. How the aedeagus passes from its right, lateral position in the terminalia to the ventral position (which is the normal adult condition) he does not describe. Nor does he satisfactorily explain how the anus, after stage (3), returns to the rearwardly directed position.

(3) *A comparison of Specimens I and II with Crampton's series and their interpretation as developmental stages*

In so far as tergite 9 is rotated through 180° relative to the normal position, so that the aedeagus and cerci point upwards instead of downwards, Specimen II (Text-fig. 5) approximates to stage (2) in the scheme, though in other respects (except the pregenital plate) it is a normal specimen of *S. hirtipes* var. *orchidea* Bött.

Specimen I may be compared with stage (4). The ninth segment is folded forward into the right side of the terminalia as a result of the curious construction of the posterior region of synsternite 7–8 (Text-fig. 1A). This region has already been described as triangular in outline when viewed from above, with an exposed posterior surface and a right lateral border in contact with tergite 9. We have also seen that the posterior region of a normal synsternite has right and left lateral surfaces, and a posterior border (Text-fig. 2). In Specimen I, however, the right lateral surface is suppressed, so that what was the left lateral surface shifts to become the posterior surface of the synsternite. What was the posterior border has moved to the right and now forms the right lateral border of the triangle (*CD*, Text-fig. 1A). This is one of the crucial points in the structure of the terminalia of this specimen, for once this asymmetrical development of a typically symmetrical structure has occurred, the segments following the synsternite are necessarily thrown to the right of the longitudinal axis of the abdomen, so that the terminalia assume the curvilinear form, that is to say, tergite 9 and its associated structures (the hypopygium) turn through 90° in a median plane. This construction corresponds to stage (3).

Synsternite 7–8 is slightly displaced to the left of the longitudinal axis of the abdomen (Text-figs. 1, 3C). Whether this has any connexion with the circumversion of tergite 9 (if there has indeed been any 'circumversion' at all in Specimen I) is not known. It is perhaps significant, however, that the sclerite is displaced to the left, and not to the right, of the longitudinal axis of the abdomen, a fact which is in accordance with the theory of the development of the terminalia described in this paper.

We must now consider the mode of attachment of tergite 9 to the right lateral border of synsternite 7–8 (*CD*, Text-fig. 1). The plane of attachment (*AB*, Text-fig. 3C) is slightly inclined at about 20° to the dorso-ventral axis of the abdomen (*CB*, Text-fig. 3C). In a normal specimen (Text-fig. 2), the mid-dorsal point of the posterior border of the synsternite (*F*, Text-fig. 2) is directly in line with the mid-dorsal point of the anterior border of tergite 9 (*H*, Text-fig. 2), that is to say, both points are on the antero-posterior axis of the abdomen (*AB*, Text-fig. 2). Referring now to Text-fig. 1, it is clear that the mid-point of the right lateral border of the synsternite (*F*, Text-fig. 1A, D) is not opposite the mid-point of the articular border of tergite 9 (*H*, Text-fig. 1D), as one would expect it to be if the relationship of the synsternite and tergite 9 were normal. If the total suppression of the right lateral surface of the posterior region of the synsternite were the only developmental peculiarity, one would expect the points *F* and *H* (Text-fig. 1) to remain in line, in spite of tergite 9 and its associated structures (the hypopygium) being turned through 90° in the horizontal plane. Since point *F* is not in line with point *H* (Text-fig. 1), it is clear that tergite 9 has rotated, relative to the synsternite, through 90° in a clockwise direction, in the plane of attachment of the synsternite and tergite 9 (*AB*, Text-fig. 3C). This condition may be regarded as one of the intermediate conditions of stage (4). The condition of the terminalia in Specimen II seems, therefore, to have arisen as a result of two main twists of the terminalia in two planes roughly at right angles to each other.

The construction of the terminalia of Specimen I has been discussed as if it represents a stage in the pupal development of the normal adult terminalia. If this is so the rotation and displacement during development of the various parts described above will actually take place in directions precisely the reverse of those described. Tergite 9 will revolve in an *anticlockwise* direction about the right lateral border of synsternite 7–8 (in the plane *AB*, Text-fig. 3C), and the posterior region of synsternite 7–8 will eventually form into a *symmetrical* structure by *developing* a right lateral surface, so as to swing tergite 9 and its associated structures around to the *left* (through 90°) into the longitudinal axis of the abdomen. Concerning the latter point, Hardy (1944) evidently had some such idea in mind when he wrote that after stage (3) 'the segments of the curvilinear terminalia reformed themselves back to the rectilinear type'.

(4) *A note on the internal anatomy of Specimens I and II*

Special care was taken in both specimens to determine the relations of the genital duct and the alimentary canal as the dissection of the terminalia proceeded, but this was a difficult task as the specimens had not been preserved in a liquid medium. The ordinary method of maceration with dilute KOH renders the soft internal parts transparent and fragile. Moreover, working out the relations of the gut and duct was not made easier by the fact that the external anatomy of the unfamiliar terminalia had to be studied at the same time. The relations of the gut and duct in the two specimens could not be established with complete certainty, but the following account summarizes the results of a careful study of the dissection.

In Specimen II there was a 180° torsion of the terminalia resulting in a crossing of the alimentary canal and genital duct in the plane defined by the antero-posterior and dorso-ventral axes (that is to say, the crossing is seen when the specimen is viewed from the side); there was no looping of the gut and duct. In order to derive this condition from that of a normal specimen, tergite 9 must revolve through 180° in an anticlockwise direction. This condition corresponds to stage (2). During development, tergite 9 may be regarded as having started from the condition of the tubes found in stage (1) and rotated clockwise through 180° to the condition of the tubes found in stage (2).

In the description of Specimen I it was stated that no looping of the genital duct over the alimentary canal was observed. Though this might actually have been the case, it is possible that in sorting out the parts during the dissection the relations of gut and duct were altered. It would seem likely, from the external anatomy of the terminalia, that the genital duct made an incomplete loop of 270° around the alimentary canal. In that case, the condition of the tubes in Specimen I would correspond to that seen in a stage of stage (4), and the torsion of the tubes may be regarded as having evolved from the condition in stage (1), through the conditions in stages (2) and (3), to a particular condition in stage (4).

(5) *Further interesting points in the terminalia of normal and the abnormal specimens*

Synsternite 7–8 is an interesting study in itself. Crampton's comparative work shows that sternites 7 and 8 have migrated to a dorsal position in the higher Diptera and have fused to form a composite plate. Putting aside for the moment conclusions drawn from comparative anatomy, let us consider the anatomy of the synsternite in normal *Sarcophaga*. It has been pointed out already (see under Specimen I) that the sclerite always consists of two well defined regions whose surface markings (i.e. presence of pollen and marginal bristles) usually differ markedly from each other. This in itself might suggest that the sclerite is a composite structure. The condition of the pregenital plate in the abnormal specimens lends support to this view: in both specimens only the posterior region of the pregenital plate is modified; the anterior region is entirely unchanged. Indeed, the outline of the anterior region of the pregenital plate in both forms may be superimposed on the corresponding regions (i.e. the anterior region) of a normal synsternite of the same species (see, for example, Fig. 6A, B, C, D of Specimen II). The posterior region, on the other hand, may be absent (Specimen II) or deformed (Specimen I). These differences between the two regions indicate that the synsternite does not behave as a unit, but that the two regions can be modified independently of one another. The abnormal specimens therefore provide independent evidence that the synsternite is probably composed of the fused sclerites of two segments; on the grounds of comparative morphology, Crampton has suggested that the anterior region is sternite 7 and the posterior region sternite 8.

In Specimens I and II it was seen that tergite 9 has revolved about the end of synsternite 7–8 to a greater or lesser extent; because of this their line of articulation with one another is greatly reduced. In general, weakness of the articulation of tergite 9 upon synsternite 7–8 is displayed in many genera of the higher Diptera and is compatible with the view that a rotation of tergite 9 about synsternite 7–8 occurs in all these forms. The articulation is apparently also weak, for example, in *Lucilia* R.-D., *Chrysomyia* R.-D., *Calliphora* R.-D., and other allied genera. Frequently, the dorsal region of the joint in these genera is unsclerotized. The connexion between synsternite and tergite seems to reach the limit of attenuation in *Calliphora* (*Triceratopyga* Rohd.) *calliphorides* Rohd., where the tergite is attached to the synsternite by two slender sclerotized processes only.

In both abnormal specimens it was seen that sternite 9 (including the hypandrial apodeme) is connected at some point with the articular border of the pregenital plate. This fact is of interest, since it implies that sternite 9 provides a link between the pregenital plate in front and the structures posterior to sternite 9. All the sternites of the abdomen therefore are in anatomical continuity in these specimens: starting from sternite 1, through sternites 2, 3, 4 and 5, through the asymmetrical sternite 6, through synsternite 7–8 to sternite 9. In *Calliphora* and other genera, though not in *Sarcophaga*, the series is continued through the bacilliform sclerites to the surstyli.

It is to be regretted that Crampton did not study

3

4

1

2

what he calls the 9th sternite (with the hypandrial apodeme and the posterior arms) in detail in the series of insects at his disposal. This structure, or its homologue, does not seem to be present in Syrphidae, but is probably present in all the higher Diptera. A comparative study of this sclerite in different families would be of great interest.

Crampton also ignores the structures which he calls the connecting rods or bacilliform sclerites, dismissing them with the remark that 'the connecting rods are probably merely secondarily-developed sclerites which do not occur in all the higher Diptera' (1942). It would, however, be interesting to study Crampton's series of insects in relation to these structures. The connecting rods are situated between the ventral borders of tergite 9. In Syrphidae, a pair of rods or a plate lies in this position (Metcalf, 1921). In *Musca*, the rods are probably fused to the surstyli. In *Lucilia*, a plate occurs in this position and, moreover, it is usually clothed with hairs, which suggests that it is a segmental sclerite. In some *Calliphora* this plate is massive. The condition in *Sarcophaga* also merits attention: on each side, antero-medial to the surstyli (the accessory plates) a small sclerotized area (usually oval in shape) is constantly present in the otherwise unsclerotized membrane under tergite 9. From their position these sclerotized areas appear to be homologous with the connecting rods in *Lucilia* and other genera, and possibly with the lateral thickenings of the plate found in this region in certain species of *Lucilia*, *Calliphora*, etc. Patton (1934) interpreted these small sclerotizations as sclerotized 8th spiracles, however. In *Sarcophaga*, unlike, for example, *Lucilia*, the bacilliform sclerites are greatly reduced—almost completely so—and do not connect the posterior arms of sternite 9 with the surstyli.

All the aforementioned structures—the pair of small sclerotized areas, the pair of rods and the single plate—appear to be homologous, and it seems desirable to re-examine Crampton's series with the object of deciding whether in fact these homologous structures are to be identified with a sternite (possibly sternite 9), thus making necessary a reconsideration of the numbering of the sternites proposed by Crampton. It may be noted that Crampton's 9th sternite is never haired.

IV. SUMMARY

This paper describes the terminalia of two abnormal males of the genus *Sarcophaga* Meigen (*Sarcophaga similis* Meade and *S. hirtipes* Wiedemann var. *orchidea* Böttcher) which appear to throw light on the interrelationships of the inverted, curvilinear and circumverted types of terminalia in Diptera. The specimens resemble certain stages described by Crampton (1944) in his comparative study of the morphology of the terminalia, and may perhaps represent stages in the pupal development of normal calypterate cyclorrhaphous Diptera.

From a study of the pregenital plate in these forms it seems probable that, in *Sarcophaga* at least, it represents two fused sclerites—probably sternites 7 and 8.

The author wishes to acknowledge his indebtedness to Dr L. E. R. Picken for advice in the preparation of the manuscript.

REFERENCES

CRAMPTON, G. C. (1942). *State Geology and Nat. Hist. Survey*, Pt. 6, Diptera, p. 67.

CRAMPTON, G. C. (1944). *Bull. Brooklyn Ent. Soc.* **39**, 1.

HARDY, G. H. (1944). *Proc. R. Ent. Soc. Lond.* (A) **19**, 52.

METCALF, C. L. (1921). *Ann. Ent. Soc. Amer.* **14**, 169.

PATTON, W. S. (1934). *Ann. Trop. Med. Parasit.* **28**, 579.

SCHRÄDER, T. (1927). *Z. Morph. Ökol. Tiere*, **8**, 1.

SENIOR WHITE, R., AUBERTIN, D. & SMART, J. (1940). *Fauna Brit. India, Diptera. VI. Calliphoridae*, p. 208.

EXPLANATION OF PLATE VI

Fig. 1. Specimen I seen from behind. Because of the curvilinear condition of the terminalia, the aedeagus, cerci, etc., are seen almost in a lateral view. The relations of the parts out of focus can be determined by referring to Text-fig. 3C.

Fig. 2. Specimen I. A left lateral view of the hinder end of the abdomen (slightly infero-lateral).

Fig. 3. Specimen II. Left lateral aspect of the hinder end of the abdomen. The aedeagus, cerci, etc., point upwards.

Fig. 4. Specimen II. A more detailed view of the right lateral aspect of the terminalia.

(*MS. received for publication* 10. IX. 1948.—Ed.)

NOTE ON *LINTONIUM VIBEX* (LINTON, 1899) (DIGENEA—TREMATODA)

By PETER CROWCROFT,* *Zoology Department, University of Tasmania*

(With 9 Figures in the Text)

In March 1947 I collected a number of digenetic trematodes from the gullets of *Cantherines setosus* (Waite) and *Cantherines güntheri* Macleay. Three individuals of each species were examined and two of each were found to contain four or five trematodes. The fishes are known locally as 'Leather-jackets', and were taken from the shallow coastal waters of southern Tasmania. The parasites appear to be *Lintonium vibex* (Linton, 1899) Stunkard & Nigrelli, 1930.

Yamaguti (1934) assigns his specimens from *Monacanthus cirrhifer* and *Cantherines unicornu* to *Lintonium vibex*, and remarks upon their similarity to *Steringotrema pulchrum* Johnston, 1914. He suggests that the American, Australian and Japanese forms may belong to the one species. Following his description of *Steringotrema pulchrum*, Johnston (1914) remarks: 'While I place this species in Odhner's genus *Steringotrema*, it appears to be more closely related to *Distomum vibex* Linton than to any of the three species enumerated by Odhner. *D. vibex* evidently belongs to the same genus.'

Layman (1930) describes a form which he identifies with *Steringotrema pulchrum* Johnston. Manter (1934) points out that this form should be placed in the genus *Lintonium*.

Although Stunkard & Nigrelli (1930) regard their genus *Lintonium* as closely allied morphologically with *Steringotrema* Odhner, they dispose of *Steringotrema pulchrum* in a footnote: 'According to Odhner (1928),. . . *Steringotrema pulchrum* Johnston, 1913 is identical with *Gastris consors* Lühe, 1906.' Odhner's opinion to which they refer is itself in the form of a footnote (p. 5): 'Dasselbe gilt auch von *Gastris consors* Lühe, 1906, mit der *Steringotrema pulchrum* S. J. Johnston, 1913 identisch ist; hier, wie in obigem Falle, sind die Wirtstiere der identischen Formen *Tetrodon*-Arten.'

I have not examined specimens of *Gastris consors*, but Lühe's illustration of a whole mount is reproduced (Fig. 1). Thanks to the courtesy of the Director of the Australian Museum, Sydney, I have examined one of Johnston's co-types of *Steringotrema pulchrum* (Fig. 2). Dr Ross F. Nigrelli kindly sent me a whole mount of *Lintonium vibex* from the 'smooth puffer', *Sphaeroides maculatus* (Fig. 3). A comparison of the illustrations of *Gastris consors* and *Steringotrema pulchrum* reveals differences in size and body proportions only. Odhner's view is regarded as correct.

Table 1

Parasite	Host	Host family	Locality
Lintonium vibex	*Sphaeroides maculatus*	Tetrodontidae	Woods Hole, Mass.
	Balistes vetula	Balistidae	Woods Hole, Mass.
	Monacanthus cirrhifer	Balistidae	Japan
	Cantherines unicornu	Balistidae	Japan
	C. modestus	Balistidae	Peter-the-Great Bay
	C. setosus	Balistidae	Tasmania
	C. güntheri	Balistidae	Tasmania
Gastris consors	*Tetrodon stellatus*	Tetrodontidae	Ceylon
	T. hispidus	Tetrodontidae	Queensland

Note. Some taxonomists include the Tetrodontidae within the Balistidae.

The principal differences between *Gastris consors* (syn. *Steringotrema pulchrum*) and *Lintonium vibex* appear in the position of the testes relative to each other and to the lateral body margins, together with slight differences in body proportion and the more posterior extent of the gut and vitellaria in the latter species. Physiological evidence of their close relationship is afforded by a consideration of their respective hosts and their distribution (Table 1).

Stunkard & Nigrelli (1930) suggest that as the 'swell fishes' are primarily inhabitants of warmer waters, the relatives of *Lintonium vibex* will be found, 'if at all, in species of *Sphaeroides* which occupy

* Now at the Bureau of Animal Population, Oxford University.

warmer seas'. The occurrence of *Gastris consors* in the warm waters of Ceylon and Queensland in a fish genus closely related to *Sphaeroides* is in accord with their view. It might be argued, however, that the forms most closely related to *Lintonium vibex* from the Woods Hole region are those reported by Yamaguti (1934), Layman (1930), and in the present paper. These forms, which all occur in cool waters, are identified with *L. vibex* simply because the recorded differences, using our present criteria, are not sufficient to justify specific differentiation.

Nigrelli & Atz (1943) conclude that *L. vibex* is a species specific to 'puffers' of the North Atlantic coast of America, and express the view that the infestation of *Balistes vetula* reported by Linton (1940), took place when the fish 'struggled northward to the Woods Hole region during the summer'. They state that *B. vetula*, examined in its natural habitat, has not been reported as a host for this parasite. While it seems probable that this view of the infestation of *B. vetula* is correct, it appears that the absence of the

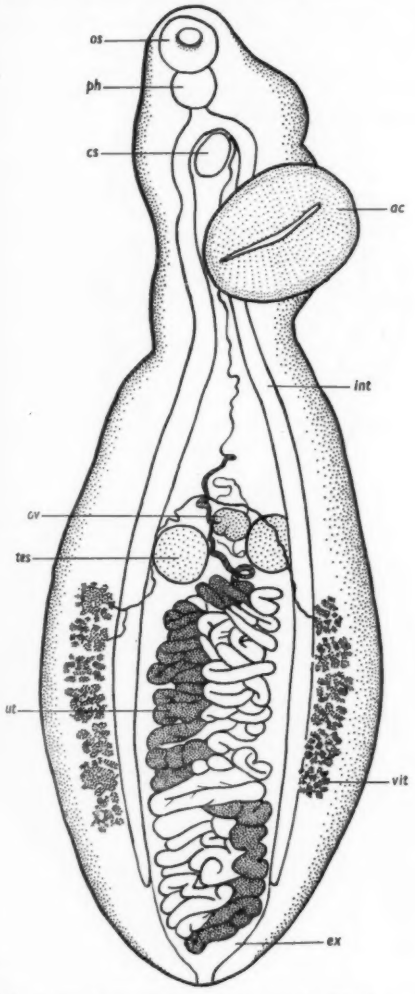


Fig. 1. *Lintonium consors* (Lühe) n.comb. Whole mount from the ventral aspect. After Lühe. Host: *Tetrodon stellatus*.

Abbreviations used in figures. *ac*, acetabulum; *cs*, cirrus-sac; *ex*, excretory vesicle; *int*, intestine; *os*, oral sucker; *ov*, ovary; *ph*, pharynx; *pp*, pars prostatica; *sem*, seminal vesicle; *sp*, spermatophore; *tes*, testis; *ut*, uterus; *vit*, vitellarium.

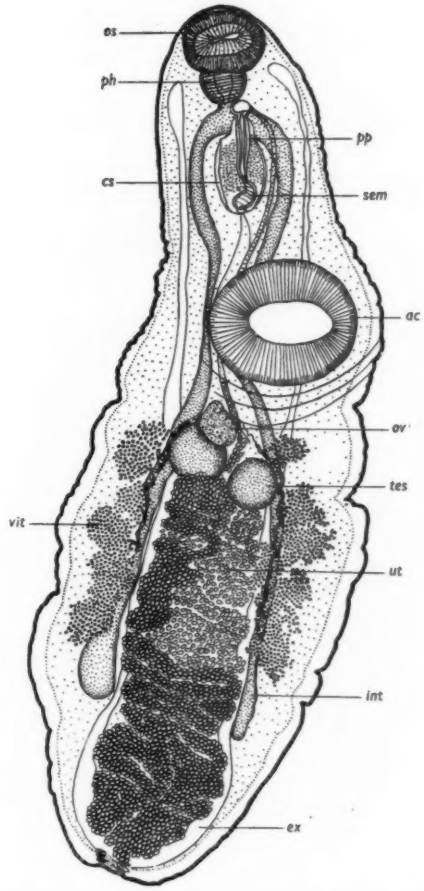


Fig. 2. *Lintonium consors* (Lühe) n.comb. (*Steringotrema pulchrum* Johnston). Whole mount, 5 mm. long from the ventral aspect. Host: *Tetrodon hispidus*.

parasite in the fish's normal habitat is not due to the specificity of *Lintonium vibex*, but rather to the existence of a temperature 'barrier' preventing overlapping in the distribution of the two animals.

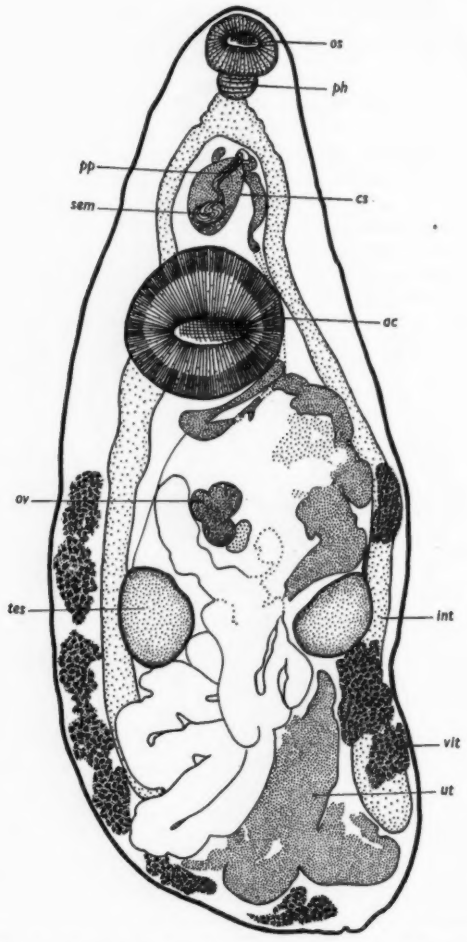


Fig. 3. *Lintonium vibex* (Linton). Whole mount, 5·9 mm. long, from the ventral aspect. Excessively flattened in mounting. Host: *Sphaeroides maculatus*.

Virtually nothing is known of the life histories and larval forms of *L. vibex* and *Gastris consors*. An examination of illustrations of immature specimens of the two species indicates the possibility of considerable differences in relative growth, and in the sequence of development of various organs. These features cannot be utilized for diagnostic purposes at the present time.

The evidence indicates that *Lintonium vibex* and *Gastris consors* cannot remain in separate genera. The genus *Gastris* Lühe, 1906 would have priority over *Lintonium* Stunkard & Nigrelli, 1930, but for the existence of the genus *Gastris* Billberg, 1820 (Lepidoptera). Thus the name *Gastris* is not available for a trematode and *Lintonium* must stand. Accordingly the new combination *Lintonium consors* is suggested for Lühe's species.

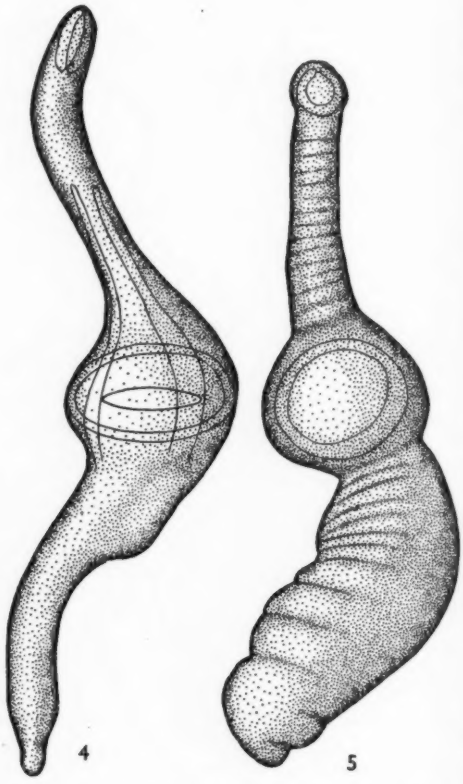


Fig. 4. *Lintonium vibex* (Linton). Small extended living specimen from the dorsal aspect. Host: *Cantherines setosus*.

Fig. 5. *Lintonium vibex* (Linton). Large contracted living specimen from the dorsal aspect. Host: *Cantherines setosus*.

Family FELLODISTOMATIDAE Odhner, 1911

Lintonium vibex (Linton)

The following notes serve principally to indicate differences between the Tasmanian form and the description of Stunkard & Nigrelli. Whole mounts

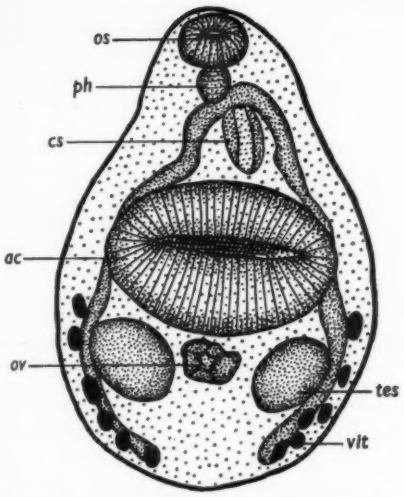


Fig. 6. *Lintonium vibex* (Linton). Whole mount, 1·57 mm. long from the ventral aspect. Host: *Cantherines setosus*.

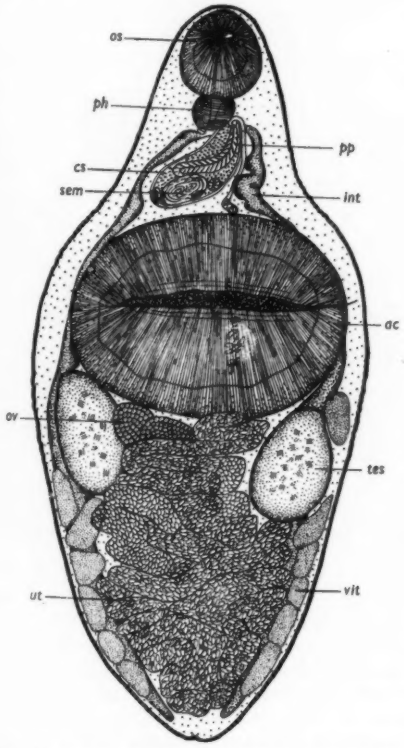


Fig. 7. *Lintonium vibex* (Linton). Whole mount, 3·27 mm. long, from the ventral aspect. Host: *Cantherines setosus*.

were stained with alum carmine and serial sections with Ehrlich's haematoxylin and eosin.

External characters. Two living unmounted specimens are shown in Figs. 4 and 5. The general form and body proportions of compressed specimens can be noted from the illustrations of three whole mounts of successively larger individuals (Figs. 6–8). The

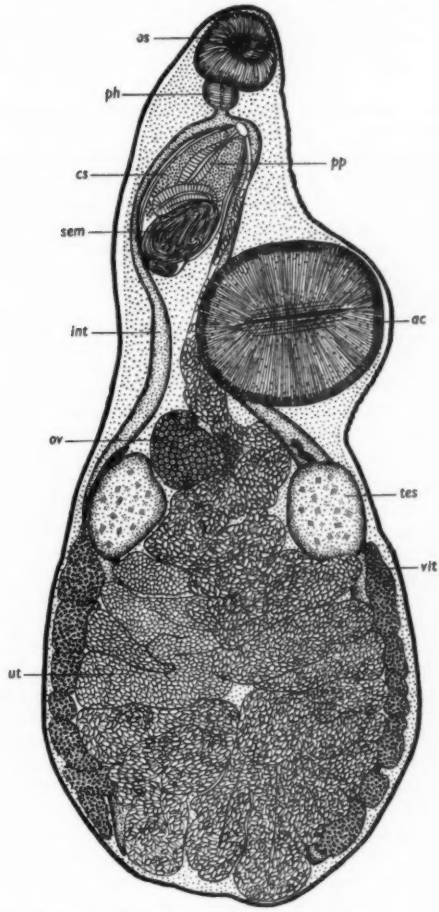


Fig. 8. *Lintonium vibex* (Linton). Whole mount, 3·89 mm. long, from the ventral aspect. Host: *Cantherines setosus*.

principal dimensions of six whole mounts are set out below (Table 2). Where two dimensions are stated for an organ, the measurement in the direction of the animal's longitudinal axis is given first. The forebody is defined as the region between the anterior extremity and the anterior edge of the acetabulum. The dimensions are of little value *per se*, but they are

Table 2

Measurements in mm.

Length	1·09	1·57	2·89	3·27	3·32	3·89
Breadth	0·71	0·98	1·34	1·52	1·25	1·74
Forebody	0·37	0·50	0·60	0·85	0·97	1·00
Oral sucker	0·14 × 0·17	0·21 × 0·26	0·32 × 0·40	0·37 × 0·40	0·40 × 0·40	0·32 × 0·37
Acetabulum	0·40 × 0·57	0·52 × 0·73	0·95 × 1·25	0·95 × 1·30	0·75 × 1·03	0·73 × 0·88
Cirrus sac	0·13 × 0·06	0·23 × 0·17	0·40 × 0·22	0·53 × 0·18	0·37 × 0·22	0·78 × 0·38
Testes	0·20 × 0·14	0·42 × 0·20	0·45 × 0·33	0·53 × 0·37	0·50 × 0·35	0·53 × 0·37
Ovary	0·08 × 0·08	0·15 × 0·25	0·23 × 0·37	0·22 × 0·40	0·25 × 0·27	0·38 × 0·38
Eggs	absent	0·031 × 0·013	0·029 × 0·015	0·031 × 0·018	0·026 × 0·018	0·037 × 0·022

useful indicators of the relative proportions and relative growth of the organs.

Alimentary system. Stunkard & Nigrelli report that the single tube connecting the pharynx with the gut rami is lined with digestive epithelium similar to that of the gut, and on this account cannot be considered a true oesophagus. In the Tasmanian form the oesophagus is extremely short and appears to be lined by an inward extension of the cuticle.

Excretory system. The small excretory vesicle and two main collecting tubes are similar in form and position to those of the American specimens.

Reproductive system. Male. The testes are situated laterally and symmetrically. Yamaguti (1934) describes the testes of the Japanese form as 'situated a little obliquely tandem at the posterior of the middle third of the body, the right being a little anterior to the left'. It may be found that this form corresponds more closely to *L. consors* n.comb. Unfortunately, no illustration is supplied, and I have been unable to correspond with Yamaguti. With reference to the seminal vesicle, Stunkard & Nigrelli state: 'The vesicle is somewhat coiled, and in some whole mounts gives the appearance of being composed of two parts.' In my specimens the bipartite nature of the seminal vesicle is quite evident. Serial sections reveal a narrow sphincter separating the small vesicle in the base of the cirrus-sac from the adjacent larger vesicle which opens into the pars prostatica. The larger vesicle may be emptied independently (Fig. 9). The whole mount of *L. vibex* supplied by Dr R. F. Nigrelli also possesses a bipartite vesicle (Fig. 3). It is interesting to note the existence of a stalked spermatophore in this species (Fig. 9). This structure appears similar to that of *Haplocladus typicus*, which is illustrated by Odhner (1911). Odhner also mentions the formation of a similar spermatophore in another Fellodistomid, *Steringophorus furciger*. It seems probable that, as the known facts about the Digenea expand, further reports of the presence of these structures will come to hand. They have been reported from two genera of the Prosorhynchidae by Odhner (1905) and Crowcroft (1946). These workers both express the opinion that the spermatophores are formed by the glands surrounding the genital atrium. Odhner (1911), however, states: 'Es ist dies um so wahrscheinlicher, als die Bildung der Spermatophorenhüllen unzweifelhaft von der Pars prostatica ausgehen muss und nicht, wie ich früher annahm, von ins Genitalatrium ausmündenden Drüsen. Solche fehlen nämlich bei der Steringophoriden vollständig, während dagegen die Pars prostatica...eine auffallende Ähnlichkeit in der Beschaffenheit ihrer Secrete mit derjenigen der betreffenden Gasterostomiden zeigt.' In *Lintonium vibex* the stalk of the spermatophore clearly originates within the pars

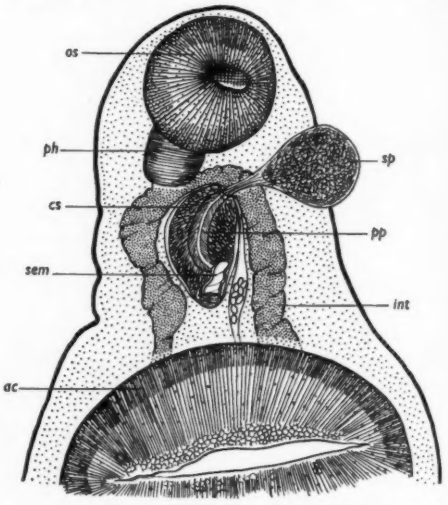


Fig. 9. *Lintonium vibex* (Linton). Forebody of whole mount, 3·32 mm. long, from the ventral aspect, showing stalked spermatophore. Host: *Cantherines setosus.*

prostatica, which must play a major part in its production. It seems likely that Odhner's latter opinion is correct, although it is possible that the formation and composition of the spermatophores is not entirely similar in the two families.

Female. The female complex corresponds to the account of Yamaguti (1934). There appear to be minor differences between my specimens and those of Stunkard & Nigrelli. First, these authors state: 'There is no seminal receptacle.' In the Tasmanian

and Japanese forms there is a small expanded chamber at the junction of the oviduct and Laurer's canal. As Yamaguti points out, this merely represents an expanded proximal portion of Laurer's canal. The proximal loops of the uterus constitute the principal receptaculum seminis. Secondly, Stunkard & Nigrelli remark: 'The oviduct...just after entering the oötype, gives off Laurer's canal.... After the origin of Laurer's canal, the female duct... receives a common vitelline duct and then turns dorsad and anteriad, to open into the uterus.' In my specimens Laurer's canal and the common vitelline duct enter the oviduct before it expands slightly into the oötype, and this is immediately followed by the uterus.

The above work was mainly carried out during the tenure of a Commonwealth research grant. I should like to express my thanks to Dr H. A. Baylis for advice and assistance in the preparation of the paper, and to Prof. V. V. Hickman for his kindly co-operation.

REFERENCES

CROWCROFT, P. W. (1946). The anatomy of two digenetic trematodes from Tasmanian food fishes. *Proc. Linn. Soc. N.S.W.* 108–18.

JOHNSTON, S. J. (1914). On some Queensland trematodes with anatomical observations and descriptions of new species and genera. *Quart. J. Micr. Sci.*, N.S., **59**, 361–400, pls. 22–7.

LAYMAN, E. M. (1930). Parasitic worms from the fishes of Peter-the-Great Bay. *Bull. Pacif. Fish. Res. Sta.* **3**, 1–120, 14 pls.

LINTON, E. (1899). Fish parasites collected at Woods Hole in 1898. *Bul. U.S. Fish. Comm.* **19**, 267–304, pls. 33–43.

LINTON, E. (1940). Trematodes from fishes mainly from the Woods Hole region, Massachusetts. *Proc. U.S. Nat. Mus.* **88**, 1–172, pls. 1–26.

LÜHE, M. (1906). Report on the trematode parasites from marine fishes of Ceylon. *Pearl Fish. Rep., Roy. Soc. Lond.* (v), pp. 97–108.

MANTER, H. W. (1934). Some digenetic trematodes from deep-water fish of Tortugas, Florida. *Publ. Carneg. Instn.* no. 435, pp. 257–345, 15 pls.

NIGRELLI, R. F. & ATZ, J. W. (1943). Biometry of puffers and their parasites. *Zoologica*, **28**, 1–8.

ODHNER, T. (1905). Die Trematoden des arktischen Gebietes. *Fauna Arctica*, **4**, 291–375, 3 pls.

ODHNER, T. (1911). Zum natürlichen System der Digenen Trematoden. III. *Zool. Anz.* **38**, 97–117.

ODHNER, T. (1928). Weitere Trematoden mit Anus. *Ark. Zool., Stockh.*, **20** (B 2), 1–6.

STUNKARD, H. W. & NIGRELLI, R. F. (1930). On *Distomum vibex* Linton, with special reference to its systematic position. *Biol. Bull. Woods Hole*, **58**, 336–43.

YAMAGUTI, S. (1934). Studies on the Helminth fauna of Japan. Part 2. Trematodes of fishes. I. *Jap. J. Zool.* **5**, 249–541.

(*MS. received for publication* 8. IV. 1949.—Ed.)

CRITHIDIA FAMILIARIS N.SP. IN *CENAEUS CARNIFEX* FABR. (HEMIPTERA)

By ALFRED J. GIBBS, *Honorary Research Assistant*

From the Department of Zoology, University of Cape Town

(With 15 Figures in the Text)

INTRODUCTION

In a paper entitled '*Crithidia* sp., parasitic in the alimentary tract of *Cenaeus carnifex*' (Gibbs, 1942), an attempt was made to describe a flagellate found in the mid-gut, hind-gut and rectum of a plant-feeding insect which is common in the Cape Peninsula, South Africa. It was shown that leishmanial forms and bodies described as mould-spores were carried around attached to the flagella of leptomonads. It was also observed that, in some form, the parasite was resistant to desiccation, as nymphs became infected when allowed to feed on the dried dejecta of infected insects. Large, fast-moving leptomonads were described as being the only forms found in the mid-gut, while smaller leptomonads with the attached bodies, as well as crithidial forms, were found in the hind-gut. The rectum was not studied on account of the difficulty then experienced in removing it unruptured.

However, further studies of the flagellates found in *Cenaeus* showed that they actually belonged to two distinct genera. One of these, *Leptomonas* sp., represented by the long leptomonads occurring in the mid-gut and by some of the smaller leptomonads in the hind-gut, is not described here. The other, a *Crithidia*, forms the subject matter of the present paper.

As regards the adherent bodies, previously stated to be mould-spores, they are now shown to be, in fact, resistant forms which are disseminated with the dejecta of the insects.

FORMS IN THE HIND-GUT AND RECTUM

The life-cycle of the flagellate was studied in experimental and natural infections of nymphs of *Cenaeus carnifex*, both in the living state and after fixation and staining. This organism is never found in the mid-gut at any stage but the infection commences in the bulbous portion of the hind-gut. Although it later spreads throughout the hind-gut and to the rectum, the parasites are most frequently found and are most numerous in the bulbous portion.

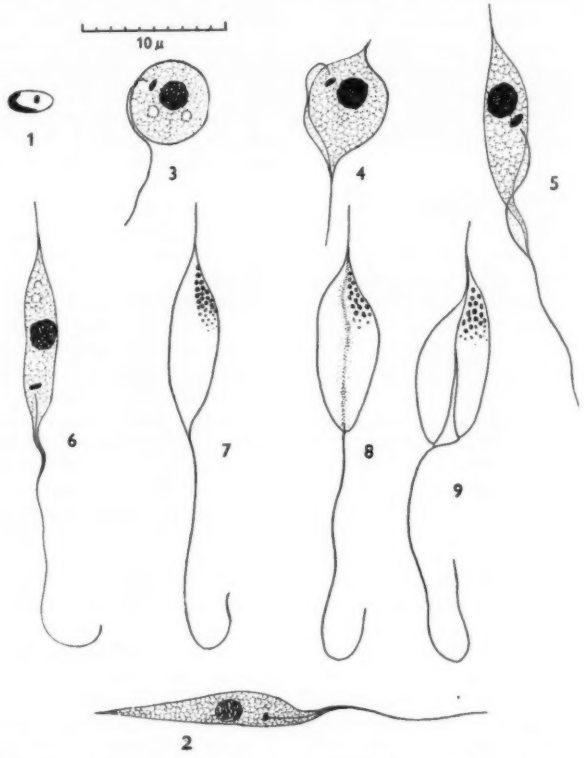
The earliest forms found in this region are represented by the resistant bodies taken up by the nymph. They are similar in appearance to the bodies found in the rectum of the adult insect and previously considered to be mould-spores. The infective bodies are ovoid and measure about 3·2 by 1·6 μ, and in section across the long axis they are narrow and slightly concave-convex. In the mature state each body is covered by an ectoplasmic pellicle, which may take on a pinkish tinge when dry-fixed and stained by Leishman's or Giemsa's method, while the internal structure is almost unstainable. However, prior to the development of a protective envelope the internal contents of the body can be readily revealed by the Romanowsky stains. It is then seen that the nucleus lies diagonally across the body at one end, while the kinetoplast is situated towards one side, approximately midway between the nucleus and the opposite end of the body (Fig.1). The staining reactions of the bodies following wet-fixation will be described later.

In the gut contents of the nymphs the internal structure of the resistant bodies stained strongly after dry-fixation, probably indicating that the protective envelope had been absorbed or dissolved. The earliest stages of flagellation were observed in one case, in which were found a large number of bodies, both ends of which had become somewhat pointed. In the course of 1 hr. they commenced to produce flagella and, when the flagellum had grown to about one-quarter of the body length, they became motile, the flagellum exhibiting a periodic flicking from side to side with an appreciable interval between each movement. This development was reminiscent of flagellating metacyclic forms of *Leptomonas ctenocephali* (Gibbs, 1947, 1948). The movement of the flagellum became progressively more vigorous with growth and, when it attained a length equal to that of the body, it waved about evenly, although there was little travel. Leptomonads measuring about 15 μ in length were later found in experimentally infected nymphs (Fig. 2).

It was not found possible to study the complete life-cycle of the parasite in the organs of the nymphs, as their maintenance under laboratory conditions

proved very difficult. Observations of the remaining phases were made from naturally infected adult insects. In the adult insect the parasite is found in three forms, viz. rounded bodies possessing active flagella, crithidials, and leptomonads which, by a special division, produce attached leishmania-like forms that later become resistant bodies. The origin of the rounded flagellates is not known. They are not, apparently, derived from leishmanials as, apart from the attached forms, leishmania-like parasites of stumpy leptomonads which develop from the crithidials.

The rounded flagellates measure about 5–6 μ across, the nucleus is eccentrically situated, while the kinetoplast lies close to the border of the body. The flagellum issues from the periphery at the point nearest to the kinetoplast and runs around the body for a distance equal to about one-third of its circumference before becoming free (Fig. 3). Later the body is drawn out to a point where the flagellum



Figs. 1–9. *Crithidia familiaris* n.sp. (×2150). Fig. 1. Resistant body. Fig. 2. Leptomonad form derived from resistant body. Fig. 3. Rounded flagellate. Figs. 4, 5. Crithidial forms. Fig. 6. Leptomonad form. Figs. 7–9. Early stages of division leading to formation of attached body, and showing granular inclusions in parent-flagellate (unstained).

have never been found in the gut. (The leishmanial clusters described in the previous paper were doubtless contaminations, from the rectum, of the concomitant leptomonad which is found in a leishmania-like form in that organ.) The sequence, however, seems clear: the resistant bodies develop directly into leptomonads (Fig. 2), then rounded motile forms appear which evolve into crithidials, while the production of the resistant bodies (the final phase in the gut) is the result of an unequal division becomes free, while that part which previously adhered to the surface now becomes the outer margin of an undulating membrane and a short, stumpy crithidial form is formed (Fig. 4). The body then elongates, the anterior tip becomes wavy, tapering gradually to a point, while the posterior end narrows abruptly but terminates in a very long thread-like filament. The nucleus is located near the posterior extremity, while the kinetoplast is situated either laterally or just anteriorly to the nucleus. The

length of the flagellum in the fully developed crithidial form approximately equals that of the body (Fig. 5). During the stage when the formation of resistant bodies is commenced the majority of the flagellates are crithidials, but the type of division leading to the formation of these attached bodies is found only among leptomonads about 12μ long with flagella from 1 to 1½ times the body length. Later, when practically all of the attached bodies are mature and ready for dissemination, crithidials are absent from the gut, and only leptomonads (nearly all with attached bodies) are found. It appears to be a reasonable assumption, therefore, that the kinetoplast of the crithidial parasite moves anteriorly, the undulating membrane is lost and leptomonads are evolved (Fig. 6), which then commence the division resulting in the production of attached bodies.

DIVISION RESULTING IN PRODUCTION OF ATTACHED BODY

An almost constant characteristic of the leptomonad flagellates, when examined in the living state, is the presence in the posterior part of the body of a number of brilliant granules. As far as could be observed in living material, the first indication of the peculiar type of division is a broadening of the body, after which the posterior granules gather towards one side, the opposite side being strongly contrasted by being free of inclusions and appearing to be smooth and glassy (Fig. 7). Then a longitudinal depression is formed in the cytoplasm (Fig. 8) and cleavage commences from the anterior end, resulting in the formation of two daughter-individuals. One of these maintains its connexion with the flagellum of the other one (which will be referred to as the 'parent flagellate') by a fine thread-like anterior process (Fig. 9). As the two flagellates separate, the connecting thread of one of them moves along the flagellum of the other, drawing its body in an anterior direction and away from the body of the parent flagellate (Fig. 10). When cleavage has extended to the posterior end, the corresponding extremity of the former individual is held to the body of the latter by a fine elastic thread, which gradually elongates, allowing the body to slide anteriorly in relation to the parent flagellate. Finally, this thread breaks, releasing the body of the daughter-individual, which is now attached to the flagellum of the parent flagellate by the anterior thread already described (Fig. 11).

In fixed and stained preparations the glassy appearance of the developing adherent body is not apparent, the cytoplasm appearing almost identical with that of the parent flagellate. A period of 1½ hr. is required to complete cleavage when the organism is studied in saline but probably under natural conditions the process would be completed in a shorter time.

At first the daughter-cells are similar in shape, both having attenuated posterior tips ending in fine filaments; the parent flagellate, however, retains the cytoplasmic inclusions seen in the living state, while these are absent in the attached body. The most marked distinction between the two cells is that one has no flagellum; it is not considered that the anterior thread connecting the non-motile form to the parent flagellate is actually a minute part of a flagellum, although it issues from a point near which the flagellum would emerge from the body. It is, first, finer than the flagellum and, secondly, even on slides on which the flagella are particularly sharply stained, there is no trace in the attached body of an intracytoplasmic portion of the axoneme, or rhizoplast.

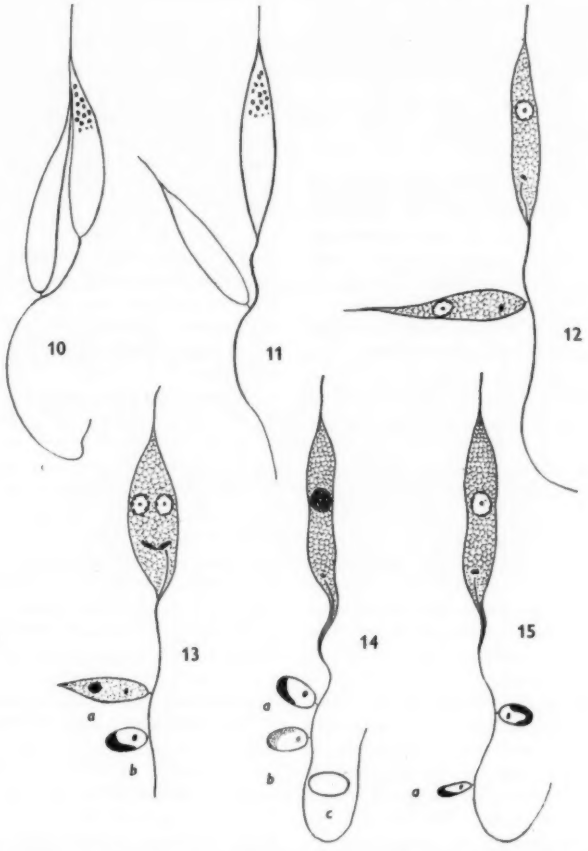
When wet-fixed, the nucleus of the attached body is, at first, identical in structure with that of the parent flagellate, being vesicular with a central karyosome (Fig. 12), but it soon becomes more compact and later appears as a solid block of chromatin. Prior to, and including this stage of development, the attached bodies react to osmotic pressure differences.

The attenuated posterior tip is soon lost and the body gradually takes on a symmetrical, ovoid outline, becoming progressively more compact until it measures about 3·2 by 1·6μ (Fig. 13*a*,*b*). The nucleus then lies obliquely across one end of the body and is bent U-shape, one side being slightly longer than the other. This appearance is identical both after dry- and wet-fixation, although in the latter case there is a slight shrinkage of the body. Following this stage the internal contents become more difficult to stain after dry-fixation, until finally, when invested in a somewhat impenetrable protective pellicle, it is difficult to demonstrate the internal structure. It is possible, however, to stain the interior of the body at any stage with Ehrlich's haematoxylin following fixation in Schaudinn's solution. The mature resistant body is unaffected by osmotic pressure differences and the enveloping pellicle exhibits a strong affinity for eosin.

In the description of the division resulting in the formation of the resistant body it was shown that, even before the breaking off of the posterior connecting thread, the non-motile cell tends to move towards the free end of the flagellum of the parent flagellate, and this direction of travel continues after separation is complete. By the time the attached body has moved approximately to the middle of the parent flagellum, when it is still considerably larger than its final mature stage but has no longer an attenuated posterior tip, the parent flagellate starts dividing again, with the formation of new attached bodies. Sometimes as many as five such bodies, at various stages of development, are found attached to the flagellum of the parent flagellate. However, as far as can be judged by their stage of development,

they are not always attached to the flagellum in the order in which they have been produced. They are never found free in the hind-gut. Figs. 14 and 15 depict flagellates with attached bodies at different stages of maturation.

more specialized division all subsequent divisions are of this type. In the case of division which results in identical flagellates, the posterior cytoplasmic granules become equally distributed between the two daughter-individuals.



Figs. 10–15. *Crithidia familiaris*, n.sp. (×2150). Figs. 10, 11. Later stages of division leading to formation of attached body (unstained). Figs. 12–15. Leptomonads with attached bodies in different stages of development. Fig. 12. Leptomonad with newly formed attached body (stained with Ehrlich's haematoxylin). Fig. 13. Leptomonad with (*a*) attached body becoming more compact in form, and (*b*) mature resistant body; (Ehrlich's haematoxylin). Fig. 14. Leptomonad with three attached bodies: (*a*) before formation of protective pellicle, (*b*) with partially developed pellicle resulting in poor staining of internal structures, and (*c*) mature body covered with pellicle impenetrable to stain, leaving the interior structure colourless (dry-fixed). Fig. 15. Leptomonad with two mature resistant bodies, one of which (*a*) shows side view (stained with Ehrlich's haematoxylin).

It is of interest to note that the more common form of binary fission resulting in the formation of two identical leptomonads may be found taking place concurrently with the type of division described above, but when once a flagellate has undergone the

Numerous leptomonads with the adherent bodies find their way to the rectum, where they attach themselves to the walls by the flagella. In the rectum the flagellates exhibit signs of degeneration, often being excessively vacuolated, and not infrequently

found dead. As many as seven resistant bodies have been found on flagellates in the rectum, where they are nearly always in the mature state. It has been observed that the threads connecting the bodies to the parent flagellates are often longer in the rectum than elsewhere. Presumably they weaken and stretch prior to the complete detachment of the bodies from the flagellates. Large masses of free resistant bodies are often present in the rectum and in the faeces, whereas crithidial parasites are never found in the rectum at any time.

TRANSMISSION

Owing to the feeding habits of the host, infection experiments on clean-bred nymphs are attended with some difficulties. In captivity they apparently do not feed until after the first moult, and it is only with difficulty that they can be brought through the second moult, after which they become sap feeders. Since the eggs are laid beneath the surface of the soil it is probable that, under natural conditions, the earlier nymphal stages are spent underground. After the first moult laboratory-hatched nymphs can be observed feeding on moist, decaying matter in the soil. As the female bug possibly contaminates the soil in the vicinity of the eggs and as its dejecta frequently contain large numbers of resistant bodies, it is likely that the infection is often acquired before the insect commences to feed on plant sap. Practical infection experiments have shown that desiccation for a reasonable period does not affect the viability of the infective forms. Washed eggs were hatched in sterilized soil in a clean container, the soil was then contaminated with the contents of the hind-gut of insects showing large numbers of resistant bodies which had been allowed to dry for over 48 hr. After the first moult a number of nymphs were observed to be infected. This experiment was repeated three times, in each case with similar results, the average proportion of infected nymphs being one in three. Resistant bodies are found early in the life cycle, so that the experiment could not be repeated with material reasonably certain of containing only flagellates, except in the case of young nymphs in which the organisms are so few in number that the result would have been inconclusive. A series of tests was made, however, with hind-gut flagellates of the concomitant organism, which is represented by leptomonads exclusively. The result was negative in every case, showing that, at least in this organism, the leptomonad form is incapable of infecting a fresh host after being subjected to desiccation.

A study of the available literature failed to bring to light any instance of flagellates being capable of bringing about transmission after being dried. Flagellate forms of *L. ctenocephali*, for example, become non-viable if allowed to dry in the faecal droplet of the flea, while the metacyclic forms, although not protected by a cyst-wall, can withstand desiccation for 2 months (Gibbs, 1947, 1948). Becker (1923*b*) has shown, by ingenious experimental infections, that flagellate forms of *Herpetomonas muscae-domesticae* are infective when the material is not allowed to become dry. Although he describes cysts in the rectum, he concludes that there is no obligatory cycle which must be completed before the parasites of one host are infective to another. In the case of flies it would appear that transmission by motile forms might frequently take place, since flagellates deposited with faecal matter on the moist surface of meat might remain active for some time in the isotonic juices and be ingested by fresh flies.

It was pointed out in a previous paper (Gibbs, 1942) that the bugs (*Cenaeus carnifex*) feed on their own dead, even under natural conditions. It has also been found that even after a few hours of captivity the insects will attack each other, the attacker mounting on the back of its victim and inserting the proboscis in its body. It has been found experimentally, that the contents of the gut of a living insect can be drawn off in this manner. Both the attacker and the victim were examined on several occasions and it was found that if the gut contents of the attacked insect contained flagellate or resistant forms of the parasite described in the present paper, these would also be found in the mid-gut of the attacking insect. The attacked insect soon dies. Except in the above circumstances, no form of the described parasite has ever been found in the mid-gut. Becker (1923*a*) believes that the cyst forms which Patton (1908) and Porter (1909) found in the crop of species of *Gerris* were in reality degenerated flagellates or yeasts. He describes cysts in the rectum but says: 'Whether or not this is the infective stage has never been experimentally proved under conditions which would eliminate all other forms of the parasite.' In this case, he was obviously not referring to forms which had been dried, as the host, the water strider, defecates on the surface of water and in damp situations. Since infected *Cenaeus carnifex* were collected from a large number of completely diverse food-plants, and microscopical examination of the plant juices never revealed any flagellates, there can be no doubt that the plant does not represent an alternate host but the parasite in question is restricted to the insect.

DISCUSSION

Since the flagellate in question passes through a definite crithidial stage but does not attain the trypanosome stage, it qualifies for inclusion in the genus *Crithidia*. The peculiar method by which the

resistant infective bodies are produced and the means by which they are prevented from being prematurely swept away from the gut of the host have not, as far as can be ascertained, been previously recorded or described. On account of this, the flagellate is referred to a distinct species, for which the name *Crithidia familiaris* n.sp. is proposed.

Definition of the new species

Crithida familiaris n.sp., is a parasite in the hind-gut and rectum of the plant-feeding *Cenaeus carnifex* (family Pyrrhocoridae, order Hemiptera) in South Africa. It is represented by three stages—crithidial, leptomonad and leishmania-like.

The crithidial forms have a maximum length of 19μ (when dry-fixed) with a free portion of the flagellum equal to the body length. Posteriorly the body is drawn out in a long, fine filament.

The leptomonad forms have a maximum length of 19μ (dry-fixed), the flagellum being 1½ times as long as the body. The posterior end of the body is drawn out in a fine filament, while the anterior end is attenuated and wavy. The nucleus of both crithidial and leptomonad forms is situated in the centre or posterior end of the body.

The leishmania-like forms, or resistant bodies, measure about 3·2 by 1·6μ. When dry-fixed the internal structure is stainable or not, according to the degree of their maturity. These forms are produced by a special form of unequal division of the leptomonad flagellate, to which they remain attached until they reach maturity, becoming free in the rectum only.

REFERENCES

BECKER, E. R. (1923*a*). *J. Parasit.* **9**, 141.

BECKER, E. R. (1923*b*). *J. Parasit.* **9**, 199.

GIBBS, A. J. (1942). *S. Afr. J. Med. Sci.* **7** (Biol. Suppl.), 50.

GIBBS, A. J. (1947). *Trans. R. Soc. Trop. Med. Hyg.* **40**, 495.

GIBBS, A. J. (1948). *Trans. R. Soc. Trop. Med. Hyg.* **42**, 89.

PATTON, W. S. (1908). *Arch. Protistenk.* **12**, 131.

PORTER, A. (1909). *Parasitology*, **2**, 348.

(*MS. received for publication* 3. XI. 1949.—Ed.)

BLOOD PARASITES OF EAST AFRICAN VERTEBRATES, WITH A BRIEF DESCRIPTION OF EXO-ERYTHROCYTIC SCHIZOGONY IN *PLASMODIUM PITMANI*

By P. C. C. GARNHAM

Reader in Medical Parasitology, London School of Hygiene and Tropical Medicine

(With Plates VII and VIII)

Little previous work appears to have been done on the blood parasites of the wild animals of Kenya. A few observations were made many years ago by Ross (1905), but the field otherwise has been practically untouched. The work of Hoare (1932) in Uganda, of Rodhain, Pons, Vandenbranden & Bequart (1913) and Schwetz (1931*a*, *b* and *c*, 1933, 1938) in the Belgian Congo, and of Wenyon (1909) in the Sudan, indicates the conditions prevalent in some of the neighbouring territories and in many instances it has been possible to assign organisms found in Kenya to species described by these observers. The Kenya Veterinary Department has examined very thoroughly the blood parasites of domestic animals; my own records include *Trypanosoma vivax*, *T. congolense* and *Theileria parva* from cattle; *Trypanosoma brucei* and *Babesia canis* from dogs, and *Aegyptianella pullorum* and *Spirochaeta anserina* from fowls. The fowl parasites were encountered whilst investigating outbreaks of so-called malaria in poultry. Consideration of these parasites is outside the scope of this paper; human parasites are also omitted.

My observations were made during a period of 15 years and covered most parts of Kenya colony. Although more than a thousand animals were examined, this number is not enough to allow the formulation of a definite distribution list of parasites. The evidence, however, suggests that *Haemoproteus* sp. and *Leucocytozoon* sp. are widespread amongst many families of birds and that *Hepatocystes kochi* is common in most of the lower monkeys in localities below 6500 ft. On the other hand, the incidence of species of *Plasmodium* appears to be patchy, even the avian forms being remarkably localized. The genus (*sensu stricto*) was absent in all mammals except man; it is noteworthy that *P. gonderi*, a common parasite of monkeys on the west side of Africa, is apparently absent in Kenya, even in forests where there is a conspicuous intrusion of western fauna.

It was unusual to observe more than a fragment of the life history of any parasite, although the length of my stay in the country enabled me often to make prolonged observations on interesting forms. For many years I refrained from naming these parasites, but now, after having had the opportunity of examining material from elsewhere in Africa, I have in most instances been able to attribute my findings to described forms or, following the principle of Stiles (1927), to give new names where there has been reasonable doubt as to their identity with known species.

REPTILES

(*a*) *Plasmodium pitmani* Hoare

Plasmodia were found only in two species of skinks—and in these species only in a localized area (Kodera) near river banks in South Kavirondo, about 10 miles inland from Lake Victoria. Further along the lake shore and elsewhere in Kenya the infection was absent. The characters of the infection were as follows:

The youngest forms consist of a tiny dot or strand of chromatin with a wisp of cytoplasm attached. Multiple infections of cells are common, as many as six parasites occurring in one cell. With the growth of the parasite a vacuole appears and the shape tends to become oval (Pl. VII, fig. 1) or 'torpedo-shaped'. The chromatin divides early and arranges itself in four to eight deeply staining granules on the periphery of the parasite (Pl. VII, figs. 2, 3). The result of the final division is the separation of eighteen to twenty merozoites, no longer peripherally distributed (Pl. VII, fig. 4).

The cytoplasm of the trophozoite is light in texture and not very basophilic; it may be drawn out into short processes. Pigment is present in a few localized yellow-black granules. The nucleus of the host cell remains unaltered in position, the parasite tending to curve around the oval end. Circular schizonts measure about 7 μ in diameter.

Gametocytes were numerous and were round or slightly oval, more regular in form than the asexual parasites. The macrogametocyte (Pl. VII, fig. 5) contains a dense mass of chromatin, localized pigment

and heavily staining cytoplasm; the microgametocyte nucleus is diffuse and occupies much of the parasite (the chromatin may be distributed in a number of discrete dots), the pigment is again localized. One or two tiny vacuoles may be present in the cytoplasm of a fully grown gametocyte.

Exo-erythrocytic schizogony (Pl. VII, figs. 6–9) was observed in the internal organs of two of the skinks (*Mabuia maculilabris*) infected with malaria. The forms were seen in smears made from the spleen and the heart; they were absent in liver and lung smears and they could not be distinguished in sections of the organs. The forms were rather scanty in comparison with the heavy infection in erythrocytes. The exo-erythrocytic schizonts were found in 'monocytes', that is to say, in cells with a large deeply staining oval nucleus and little cytoplasm; very often the schizont had been detached from this host cell during the process of smearing. The schizonts were larger than mature schizonts in erythrocytes, and about 14 μ across. One large form (Pl. VII, fig. 9) was spread 25 μ across the slide. The young exo-erythrocytic forms consisted of a large amount of cytoplasm and surprisingly little chromatin (Pl. VII, fig. 6), a parasite 7 μ in diameter might have only two or three pieces of chromatin, lying in tiny vacuoles. The cytoplasm of the younger forms stains a dark slatey blue with a ground glass texture; it becomes vacuolated and in the later forms it is scarcely visible as a covering on the particles of chromatin which form the merozoites. These were very small and usually numbered about thirty to fifty in a single exo-erythrocytic schizont; in the large form referred to above, there were over a hundred merozoites. Pigment, of course, was absent. The absence of exo-erythrocytic forms in the blood is in contrast to their presence in infections of *Plasmodium mexicanum* in American lizards (Thompson & Huff, 1944). Exo-erythrocytic schizogony in the African parasite appears to be solely of the gallinaceum-type.

Schwetz (1931*a*) points out the great variation in size and shape of the lizard plasmodia he studied at Stanleyville. There is a similar polymorphism in these East African parasites. Twenty infections (including a minority of very heavy ones) were studied in two species of skink, *Mabuia maculilabris* and *M. striata*. Blood slides were taken at 4-hourly intervals and infected blood was inoculated intraperitoneally into clean skinks (*M. striata* from Nairobi). One out of four of these reptiles developed a light infection. These observations confirmed the polymorphism of the parasite and also its asynchronous development in the blood stream.

The correct designation of this *Plasmodium* is doubtful. *P. mabuiae* Wenyon from the Sudan, *P. maculilabre* Schwetz from the Congo, and *P. pitmani* Hoare from Uganda, have all been described from this genus of lizard and closely resemble each other. The Kenya parasite is nearest both geographically and morphologically to *P. pitmani*. Hoare's (1932) description of the latter is not complete in that the fully mature schizont was not seen. The Kenya infections differ in that the number of merozoites was twenty instead of ten, and in the absence of vermicular gametocytes, but otherwise the resemblance was close and I think these parasites may be referred to as *P. pitmani*, now defined as a parasite of East African skinks which has a polar distribution in the unaltered erythrocyte, often showing multiple infections in erythrocytes with the chromatin of schizonts first peripheral and later collected into as many as twenty merozoites with round or oval gametocytes occupying not much more than half the cell and with exo-erythrocytic development of gallinaceum-type in 'monocytes' in the internal organs.

(*b*) *Haemogregarines*

Three types of haemogregarines were studied in cold blooded vertebrates: (1) *Hepatozoon pettiti* Thiroux emend. Hoare; (2) *H. minchini* n.sp.; (3) *Haemogregarina hamata* n.sp.

Hepatozoon pettiti was found in the blood of a 15 ft. crocodile from Lake Victoria; younger crocodiles 20 in. long did not exhibit this parasite, though Van den Berghe (1942) found crocodiles of this size parasitized in the Katanga.

H. minchini. The swamp snake *Crotaphopeltis degeni* is common in the papyrus swamps around the Kavirondo Gulf of Lake Victoria. A large proportion of these snakes harbour a haemogregarine in the blood, sometimes in very large numbers, as many as half the red blood corpuscles being infected. The nucleus of the host cell is pushed to one side but the erythrocyte otherwise is unchanged. Double infections occasionally are seen. The haemogregarines are fairly uniform in appearance, though there may be slight variations in width. The length in stained smears was 13–14 μ and the width 3–4 μ. The organism possesses the usual sausage-shaped body with a typical voluminous nucleus in hyaline cytoplasm (Pl. VII, fig. 10). Sometimes the cytoplasm was a little vacuolated and reddish granules were occasionally seen at one pole.

Schizogony of this haemogregarine takes place in the inter-alveolar septa of the lungs. The septa become oedematous and infiltrated with large numbers of lymphocytes and reticulo-endothelial cells; lying amongst these are the schizonts apparently free in the interstitial tissue and certainly not in the walls of the blood vessels. The chromatin in these schizonts stains more intensely with Romanowsky stain than any other structure I know. The usual agents fail to decolorize the nuclei, and treatment with acid alcohol is necessary to obtain differentiation.

The smallest asexual forms that were observed were oval in shape, about 10 μ long with the chromatin masses sometimes 3 μ in diameter. The chromatin at this stage was in two or four pieces, and it differed from that of the more mature parasite in having a blurred instead of a sharply defined outline (Pl. VII, fig. 11). The texture of the cytoplasm resembled a loose network. As the chromatin divides, the nuclei collect on the surface of the body where they stand out very prominently as dense spherical masses nearly 3 μ in diameter (Pl. VII, figs. 12–14). The schizont itself at this stage measures 20 μ, when fully mature it reaches 25 μ. The mature form contains the merozoites lying in roughly parallel rows (Pl. VII, figs. 15, 16), each merozoite resembling a haemogregarine. The number of merozoites is small; it is difficult to count them accurately even in serial sections, but an approximate number is twenty. The merozoites often give a fan-like shape to the schizont, the handle of the fan being represented by a large residual body. There was no indication here of the presence of two different types of schizonts with larger and smaller merozoites respectively as described by various workers (e.g. Shortt, 1917 in the haemogregarine of Indian Agamids).

The blood vessels in the lung were congested and the erythrocytes within them were heavily parasitized with haemogregarines. In sections, and after wet fixation, the haemogregarines are about 10 μ long compared with 14 μ in dry-fixed, thinly spread blood films.

Hoare (1932) described a haemogregarine in a snake of the same genus (*C. hotamboeia*) from the other side of Lake Victoria, but this organism was larger (20 μ long) and caused considerable distortion of the infected red blood cells. These characters differentiate it from the Kenya parasite. The developmental stages in the lung indicate that this haemogregarine does not belong to the *Eimeria* group (e.g. *Schellackia* or *Lankesterella*), and it is most likely to be a haemogregarine of the *Adelea* type of the genus *Hepatozoon*. It is proposed to name it *Hepatozoon minchini* in honour of E. A. Minchin who was one of the first to study these reptilian parasites (1910).

Haemogregarina hamata. A new species of haemogregarine was found in a lizard, *Lacerta jacksoni* Boulenger, caught near the Kitare river in South Kavirondo, Kenya. This is an unusual haemogregarine (Pl. VII, figs. 17–19), nearly twice the length of a normal red blood cell, 23 × 5 μ. The cytoplasm is finely granular. The nucleus is large, central, and occupies nearly the full width of the parasite. The chromatin is in coarse fragments. Heavily stained preparations reveal a capsule or thickening of the periphery (Pl. VII, fig. 17). One end of the haemogregarine is usually slightly more pointed than the other blunter extremity. The former is often bent over (Pl. VII, fig. 19), so that it looks like a crochet hook.

These haemogregarines cause much distortion and change in the infected red blood cells. The erythrocyte nucleus is flattened and elongated, whilst the yellow-staining portion of the cell is either absent or present as a small slice beside the nucleus. The cell envelope is stretched to thinness and is usually only visible on the concave surface between the two ends of the parasite, not unlike the appearance of the corpuscular envelope around a *Plasmodium falciparum* gametocyte. This parasite of the lizard is named *Haemogregarina hamata*, the generic name being applied *sensu lato*.

(c) *Trypanosoma martini* Bouet

A rather light infection of a trypanosome was found in a skink, *Mabuia maculilabris*, from near Lake Victoria, from the same place where malarial infection in these reptiles occurs. It is a monomorphic trypanosome (Pl. VIII, figs, 23, 24), curved on itself with a floppy undulating membrane which invariably causes a twisting of the posterior extremity. This extremity is blunt, the twisted outer border being flatter than the more curved inner. The nucleus is oval in shape; the minute kinetoplast is immediately adjacent to it and both structures are close to the posterior end. The main body of the trypanosome is broad and contains myonemes. There is a free flagellum. The measurements are as follows:

	μ
Posterior tip to nucleus	5
Nucleus	4
Nucleus to anterior tip	28
Free flagellum	15
Total length	52
Width	7·5
Nuclear index	0·2

Wenyon (1926) suggested that the various trypanosomes described from skinks of the genus *Mabuia* may all be one stage of a polymorphic organism; Laveran & Mesnil (1912) also commented on the pleomorphism of these same trypanosomes. The parasites described by Martin (1907), Schwetz (1931*a*, 1933*a*) and Hoare (1932), fall into this polymorphic category, and are perhaps rightly to be called *Trypanosoma boueti*. The Kenya trypanosome, on the other hand, much more resembles the monomorphic *T. martini* described by Bouet (1909) from *Mabuia maculilabris* from the Ivory Coast. He noted also how the posterior extremity makes a spiral turn on itself; the total length of his form was 73 μ. The Kenya form therefore may be referred to as a small race of *T. martini*.

(*d*) *Pirhaemocyton granosa* n.sp.

In the blood corpuscles of a lizard, *Agama colonorum*, caught in the Teita Hills, was found a curious unpigmented parasite (Pl. VII, figs. 20–22) difficult to identify with certainty but perhaps of a piroplasmic nature. It was of a rather uniform size and shape, oval and about $6 \times 3{\cdot}5\,\mu$. The maximum size was $8 \times 5\,\mu$. A few tiny forms were seen. The cytoplasm was attenuated and could only be stained with difficulty. With Giemsa stain a few dark red granules were visible along one border and a lighter staining oval or round mass on the opposite side of the parasite. Various unpigmented parasites of the erythrocytes of cold blooded animals have been described, but none of the descriptions exactly fits these parasites. They resemble most the description of *Pirhaemocyton*, particularly the species *P. lacertae* described by Brumpt & Lavier (1935); like the latter the East African forms were unaccompanied by an albuminoid inclusion in the red cell. The parasite is named *P. granosa*.

BIRDS

(*a*) *Avian malaria*

Three species of *Plasmodium* were observed: (i) *P. durae* Herman; (ii) *P. relictum* (Grassi & Feletti); (iii) *P. vaughani* (Novy & MacNeal).

Kenya is the country of origin of *P. durae*, an avian parasite of large size first discovered by J. R. Hudson and described by Herman (1941) and Purchase (1942). Its normal host is probably a wild bird; it causes outbreaks of disease amongst turkeys which are very susceptible to the infection. I have studied the parasite in these birds in the laboratory and it does not appear to me to resemble any natural infections in the wild birds that I have yet encountered.

Apart from *P. durae*, avian plasmodia were found in only two localities in Kenya, in the Teita Hills and in the forests in and around the capital, Nairobi. The same kinds of birds were examined elsewhere but without finding plasmodia. Two species of parasite were found, one large and one small. The large form resembles *P. relictum*. It was found fairly often in the weaver, *Othyphantes reichenowi*; less frequently in the sparrow, *Passer iagoensis*. The nucleus of an infected erythrocyte is displaced by the growing parasite; sometimes the half-grown schizont will loop around one pole of the nucleus. The number of merozoites varies from strain to strain—one bird producing eight, another twenty. The pigment is usually collected in the centre. The gametocytes are circular, rectangular, or occasionally dactylosome-shaped, with round pigment granules. These parasites were transmitted to canaries which developed rather light infections.

The smaller *Plasmodium* was common in the weavers of the Teita Hills; it was found also in fiscal shrikes near Nairobi. It is a very tiny parasite; young trophozoites are amoeboid and irregular in form, with the cytoplasm sometimes stretching across the cell (Pl. VIII, fig. 25). When the chromatin divides into four pieces, the parasite has rather the appearance of a *Nuttallia* (Pl. VIII, fig. 26), though pigment is present in fine grains. At this stage the parasite measures about $2{\cdot}5\,\mu$. The cytoplasm seems to disappear as the merozoites separate. There were nearly always four merozoites, though very rarely six were found. The merozoites (Pl. VIII, fig. 27) are neatly grouped at one end of the erythrocyte; each one measures about $0{\cdot}5\,\mu$ and appears hollow inside. There is usually one block of pigment at the side of the mature schizont. It was difficult to distinguish gametocytes with certainty because nearly all the infections were accompanied by a *Haemoproteus*. However, in two infections, the latter parasite was apparently absent and a number of elongate and oval gametocytes were found (Pl. VIII, fig. 28), $8 \times 3\,\mu$ in size.

Prof. Edmond Sergent examined a stained blood slide showing this small *Plasmodium*. He was of the opinion that it was definitely not *P. rouxi*. Manwell (1935) in a re-description of *P. vaughani* states that it is probable that this species will be found to have a world-wide distribution. The Kenya parasite resembles it in most respects, though the refringent pigment granules that Manwell described were not noticed. The characteristic almost complete absence of cytoplasm in the mature schizont was also noted by Giovannola (1939). Transmission experiments failed, possibly because the subinoculated birds were not examined over a sufficiently long period (Manwell states that the incubation period of this species is often prolonged for several weeks).

The small avian parasite of Kenya is provisionally determined as *P. vaughani* (Novy & MacNeal).

(*b*) *Haemoproteus*

The gametocytes of this genus of parasite could rarely be allotted specific names; their exact determination would require not only comparison with described species, but also elucidation of the asexual stages. The latter stages were sought for without success in this investigation. *Haemoproteus* was found in the following birds from many localities: *Othyphantes reichenowi*, *Colius striatus*, *Argya rubiginosa*, *Phyllastrephus strepitans*, *Streptopelia semitorquata*, *Passer griseus*, *P. iagoensis*, *Sitagra jacksoni*, *Pycnonotus tricolor*.

The blood forms were present in variable numbers, sometimes the infection was extremely heavy with numerous young gametocytes, several often inhabiting a single corpuscle. Exflagellation of the

microgametocyte was easily produced, particularly if a wet film was kept in a 37° C. incubator for 10 min.

In the pigeon the *Haemoproteus* gametocytes were often in different shapes, both sausage-shaped and as typical halteridia. In these birds the infection did not reach the blood until after the age of 3 weeks; the nestlings were always lightly infested with hippoboscid flies.

The parasite in the grey-headed sparrow (*Passer griseus ugandae*) possesses a characteristic morphology. The adult gametocyte never encircles the nucleus of the host cell. It tends to push the nucleus aside and very often there is little to be seen of the cytoplasm of the erythrocyte. The microgametocyte (Pl. VIII, figs. 31, 32) has irregular indentations of the extremities, the chromatin is arranged very diffusely throughout a large part of the body of the parasite and the pigment is concentrated in a few coarse granules at each pole. The nucleus of the macrogametocyte (Pl. VIII, figs. 29, 30) is very characteristic, lying always in a terminal position and taking the form of a small oval or triangular mass on the outer border. The pigment resembles that of the male or it may be in finer particles. In heavily stained preparations, the cytoplasm of the female is obscured by numerous purple granules, which first suggested that the organism was a schizont of a *Plasmodium*. Schwetz (1935) noticed similar granules in the *Haemoproteus* of a Congo owl, which, however, differed in other respects. The average size of the Kenya parasite is $12 \times 3\,\mu$. Sergent & Sergent (1948) pointed out the apparent identity of several unnamed species of *Haemoproteus* in sparrows of the Old World, characterized by irregularity or indentations of the border and named the species *H. wenyoni*. I think the Kenya species may also be identified by this name.

(c) *Leucocytozoon*

The gametocytes of this parasite are found nearly as commonly as those of *Haemoproteus*, and often the two infections are present in one bird. Infected birds were found in all parts of Kenya and belonged to the following species: *Uraeginthus bengalus*, *Argya rubiginosa*, *Passer griseus*, *P. iagoensis*, *Bubulcus ibis*, *Gymnoschizorhis personata*, *Othyphantes reichenowi*, *Sitagra jacksoni*, *S. capitalis*.

The organisms belong to the round type (Pl. VIII, figs. 33, 42) none of the spindle forms being observed. No attempt was made to differentiate species. *Leucocytozoon* is one of the few parasites of the indigenous animals described in this paper which was observed to have any pathogenicity. I was able to watch the progress of an enzootic in a colony of weaver birds (*Sitagra jacksoni*) in the eucalyptus trees outside my laboratory in Kisumu. Nearly all the adult birds in this colony showed *Leucocytozoon* in the blood, as a rule, in rather scanty numbers. The youngest birds, found in the nests about April each year, had no parasites in the blood or organs then, but, at about 3 weeks of age, they began falling dead out of the nests. The blood of the dead or dying birds was extremely heavily infected with *Leucocytozoon*; the spleens were much hypertrophied and contained developmental stages. A proportion survived and the intensity of the infection decreased as the birds became adult. The vector was not discovered, but was probably not a *Simulium* as this insect was thought to be absent from the locality.

Unfortunately sections of infected organs were not made, but smears, particularly of the spleen and kidney, showed the presence of innumerable tiny merozoites, and more uncommonly cytomeres (Pl. VIII, fig. 34) of a ruptured megaloschizont. These cytomeres were packed with red-staining merozoites and contained in the centre an ill-defined residual body. Their size varied considerably—the smallest were $1\,\mu$ in diameter, the largest $38\,\mu$. Their resemblance to the cytomeres of *Haemoproteus* was noticed immediately; and when the work of Huff (1942) and Wingstrand (1948) was published it became obvious what these structures represented. Pl. VIII, fig. 35 shows the appearance in a section of a cytomere from a nearly mature megaloschizont from the crow (material kindly supplied by Dr Wingstand) and the resemblance to the smear preparation of the Kenya birds is close.

(d) *Atoxoplasma*

The correct designation of certain avian parasites is discussed below. They were found principally in a chatterer, *Argya rubiginosa*, in the grounds of the quarantine hospital on Mombasa Island; they were also found in a shrike, *Lanius collaris*, in Nairobi, and in shrikes in the Kikumbuliu Reserve. In Mombasa, every *Argya rubiginosa* was infected with the parasite. The parasite occupies large mononuclear (reticulo-endothelial) cells and less often lymphocytes in the septa of the lung. Infected cells escape into the peripheral blood in small numbers, and smears of organs such as the liver and spleen may rarely exhibit parasites. They were never found in the kidney, bone-marrow, intestinal epithelium, lymph glands or peritoneal or pleural fluid. The parasites in the peripheral blood tend to inhabit lymphocytes, into whose nuclei the organisms project; sometimes the indentation is so extreme that the nucleus becomes divided into two portions. In the lung, the infected cells are mostly large monocytes with much clear cytoplasm, and there is no association between parasite and nucleus.

The parasite is usually a short sausage-shaped object with a blurred outline (Pl. VIII, figs. 36, 37); there is apparently no limiting membrane. Sometimes the body is slightly curved and the two ends

are round and equal in size. Observations on lung juice examined in the wet state failed to reveal motility in these organisms. They stain rather poorly with Romanowsky's stains. The cytoplasm stains a delicate blue and may contain one or two tiny vacuoles; the nucleus is voluminous, occupying half the parasite and contains finely granular chromatin with a small, densely staining karyosome. Parasites from the blood often shewed the nucleus stretched as a band transversely across the body. In addition to the sausage or oval forms, there are also spherical parasites with extremely large central nuclei. The average size of the oval forms is $7{\cdot}5 \times 3\,\mu$ in dried smears. In sections of the lungs, the parasites are not easily distinguished. Small round bodies by the side of monocytes can be seen, with a central nuclear mass considerably denser and smaller than that of the nuclei seen in smears.

As a rule only one parasite is found in a cell; when more than one exists, the two organisms apparently lie as far from each other as possible, usually one on each side of the nucleus. Occasionally extracellular forms (Pl. VIII, fig. 38) are seen which have obviously been detached from the host cell.

Many young birds, including nestlings just able to fly, were examined in order to find the developmental stages of the parasite. Schizogony was not observed, but in a number of infections, cells containing four or five round parasites (Pl. VIII, fig. 39) were seen, multiplication apparently having taken place by simple division.

The mode of transmission is unknown. Lice were collected from two heavily infected birds but, on dissection, no parasites were found in the gut or body cavity. Attempts to transfer the infection to other animals were unsuccessful. Heavily infected lungs were ground up in normal saline and the emulsion was inoculated intraperitoneally and intrapleurally into rabbits, and intramuscularly into guinea-pigs and canaries. None of these animals developed the infection.

This parasite has a very restricted distribution, although all the birds of one species (*Argya rubiginosa*) in Mombasa were found infected from nestling stage upwards.

I have tried to give a name to the parasites since I first saw them in 1932. In the intervening years, useful discussions on similar parasites have been made in America, notably by Manwell (1939) and by Wolfson (1940). Miss Wolfson divided her parasites tentatively into the true toxoplasmas (thought to be identical with the mammalian forms, e.g. Pl. VIII, fig. 40, and subsequently confirmed as such by Nobrega & Reis, 1942) and distinct parasites resembling certain stages of leucocytic haemogregarines. Manwell (1939) also stated that the avian and mammalian toxoplasmas are so unlike that they may well belong to different genera. Later, Manwell, Coulston, Binckley & Jones (1945) suggested that the former are probably the merozoites of a coccidian parasite of the intestine, basing this view on the co-existence of the two forms, the coccidia producing merozoites indistinguishable from 'toxoplasma'. Aragão (1933) still regards his avian organisms as haemogregarines, which he describes as motile, oval bodies $6\,\mu$ long, most commonly found in mononuclear leucocytes of the lungs. The motility of similar forms was stressed as long ago as 1904 by Novy & MacNeal.

Raffaele (1938) described four types of unpigmented parasites from the internal organs of Italian birds. The last of these resembles the parasites under discussion and Raffaele, like everyone else, was dubious in regard to their nature. He concluded by assigning them provisionally to the Toxoplasmas, although noting that Kikuth refrained from using any name. Herman (1937), in a discussion on the taxonomy of these parasites as found in sparrows in North America, came to the conclusion that the correct name was *Toxoplasma paddae* or possibly *T. gondi*, but the pictures illustrating his paper suggest that he was dealing with more than one kind of organism.

It is clear that these parasites are not toxoplasmas, if one accepts Sabin's (1939) definition of the genus as organisms which are able to multiply and produce a disease in a variety of hosts including birds and mammals. Are they then haemogregarines? A haemogregarine (e.g. *Hepatozoon adiei* Hoare, 1924) typically is motile, has a clear-cut border surrounding hyaline cytoplasm (Pl. VIII, fig. 41) and exhibits schizogony in the internal organs or vessels. All these features are missing in the parasites under discussion. The term *Leucocytogregarina* employed by Fantham (1924) for a similar parasite of a South African weaver bird is inadmissable because this name was first applied to the mouse *Hepatozoon*. Manwell's supposition that the parasites may be coccidial in nature does not appear to be true of the East African forms, because only in one infection was a coccidial infection demonstrated, and in this infection, although the organisms were very numerous in the lungs not a single one could be found in smears made from the intestinal mucosa. The latter smears showed very numerous oocysts, macrogametocytes, microgametocytes and a few schizonts of an *Eimeria*. Smears from the intestine of seven other heavily infected birds were negative both for coccidia and for the parasite under discussion.

The infection was frequently accompanied by *Leucocytozoon* and *Haemoproteus*, but like the early workers (Adie, 1908; Marullaz, 1913) I did not find this to be invariably the case and for this reason and because of the lack of resemblance of young haemoproteids (see Pl. VIII, fig. 42) to these parasites, the

possibility that the latter represent a stage in the development of *Leucocytozoon* seems improbable.

Although the systematic position of these organisms remains unknown, for the sake of convenience, it is desirable to give them a new generic name and I suggest the name *Atoxoplasma* nov.gen.; for these parasites which inhabit the monocytes of birds from many parts of the world, are strictly host specific, non-pathogenic and possess a delicately granular cytoplasm not enclosed by a periplast, and a large diffuse nucleus with a tiny karyosome. The strict host specificity and the differences in size make Marullaz's specific name of *avium* inadequate; the Mombasa parasite is, therefore, named *Atoxoplasma argyae*; *A. avium* (Marullaz) is selected as the type species.

MAMMALS

Apart from monkeys, and domestic animals suffering from various protozoal diseases, the mammals of East Africa show few parasites in their blood. I have examined with almost negative results the blood of antelopes, bush-babies (*Galago*), bats (*Eidolon helvum* and nycterids) and small rodents. *Trypanosoma lewisi* is common, however, in the wild rodents.

(*a*) *Spirochaeta harveyi* Garnham

I have described elsewhere (1947*a*) a new spirochaete of the relapsing fever type found in the blood of a *Cercopithecus aethiops* monkey. This animal was captured in the Mau Forest at an altitude of about 7000 ft. The spirochaete was named *Spirochaeta harveyi*. Dr R. Heisch informs me that the strain is still being maintained in white mice at the Medical Research Laboratory, Nairobi.

(*b*) *Hepatocystes kochi* (Laveran)

A description has been published (Garnham, 1947*b*, 1948) of the incidence and developmental asexual cycle of *Hepatocystes kochi*, a malaria-like parasite common in most of the lower monkeys in Kenya and elsewhere in Africa.

(*c*) *Hepatocystes epomophori* (Rodhain)

The blood of an adult fruit bat, *Epomophorus labiatus*, showed a fairly heavy infection of a pigmented parasite. The bat was caught in the Kaimosi Forest of West Kenya. Macrogametocytes and rings only were found. These parasites (Pl. VIII, figs. 43, 44) closely resembled the gametocytes of *Hepatocystes kochi*. The female stains the same characteristic steely blue colour with Giemsa's stain. It is a spherical body easily distorted when the blood is smeared. The diameter is slightly more than 7 μ (the uninfected corpuscles measuring about 6 μ). The cytoplasm is light in texture and may show small vacuoles; the nucleus is eccentric and is composed of an oval achromatic sac inside which lies the heavily staining karyosome. Very fine pigment is scattered throughout the cytoplasm. The fully developed gametocytes apparently lie free in the blood stream, for no trace of a cell envelope is visible. The rings are amoeboid and cause no changes in the erythrocyte. Microgametocytes and asexual forms were not seen.

It seems probable that this parasite is identical with the one described by Rodhain (1926) under the name of *Plasmodium epomophori* from bats belonging to the same genus in the Congo (from the environs of Stanleyville and other places). It is true that Rodhain described asexual forms though he noted their rarity, and it seems likely that these bodies may be similar to the 'schizonts' seen by Schwetz (1933*b*) in *Hepatocystes kochi* infections, and interpreted by me as detached fragments of a merocyst. Not only does the general morphology of this parasite resemble that of *H. kochi*, but Rodhain's experiences of subinoculation of infected blood are extremely like mine (1948) with the monkey parasite.

It is therefore proposed to transfer this parasite of tropical African fruit bats provisionally to the genus *Hepatocystes*, calling it *H. epomophori* (Rodhain). Its exact position in the Haemoproteidae will remain unknown until more details of the life cycle are discovered.

(*d*) *Babesia pitheci* (Ross)

An adult female monkey (*Cercopithecus mitis kolbi*) was captured in the Langata Forest near Nairobi in October 1946. Its blood showed a light infection of *Hepatocystes kochi* gametocytes. It was splenectomized on 14 February 1947 and 24 days later numerous piroplasms were seen in the blood. The parasites increased in number for 5 days and then became fewer. Severe anaemia developed, with anisocytosis, poikilocytosis and the presence of normoblasts. The numbers of parasites declined still further as the animal became more ill, and on the eleventh day of the attack the monkey died.

The organism was a large piroplasm occupying nearly half the erythrocyte. Three principal forms were discerned. Oval or pear-shaped bodies (Pl. VIII, figs. 45, 47) were numerous; these possessed a thick rim of cytoplasm surrounding a central vacuole with an oval, circular or U-shaped mass of chromatin. One to six parasites were found in a cell, most frequently two. They were 4–6 μ in length. Tenuiform parasites (Pl. VIII, fig. 46) were not uncommon, the cytoplasm sometimes being drawn out in fine processes throughout most of the host cell. The chromatin in these forms might be in several pieces. Lastly, circular forms (Pl. VIII, fig. 48) were seen, some as large as 4 μ in diameter, nearly always single in the

cell. In addition to the foregoing, smaller, apparently young, parasites were encountered consisting of a large chromatin dot on the margin of an oval of cytoplasm about 2 μ in length.

Parasites undergoing division (Pl. VIII, fig. 49) were observed, the process apparently being as follows: the chromatin of a pear-shaped form first divides and then longitudinal division of the cytoplasm commences at the blunt end and is completed at the point. The details of nuclear division as described by Nuttall & Graham-Smith (1908) were not observed. It was impossible to determine whether the various morphological forms of the parasite represented different stages in the life cycle; the single circular forms were very distinctive and perhaps were asexual in nature. Their relative prevalence differed during the course of the cycle; in the early part, pear-shaped forms were commonest, later the circular parasites became more numerous. Smears of liver, heart, lymph gland and kidney were made at the autopsy of the monkey. No developmental stages were seen.

On the third day of the infection 0·5 c.c. of blood were inoculated into two baby monkeys, one of the same species and the other a *Cercopithecus aethiops johnsoni*. The latter did not develop an infection but on the day following inoculation, scanty piroplasms were seen in the blood of the former and these parasites persisted in small numbers for several weeks.

It seems highly probable that this simian piroplasm is identical with the one (*Piroplasma pitheci*) discovered by P. H. Ross (1905) in approximately the same area (Kikuyu) which also caused fatal infections in *Cercopithecus* monkeys. His parasites appear to have been much smaller than mine—the largest measuring 3 μ in length instead of 6 μ, but he did not illustrate them. Diagrammatic sketches of the same parasite were made by Nuttall & Graham-Smith (1908) and, although the scale of magnification is absent, the piroplasms in their illustration occupy about the same proportion of the erythrocyte as do mine. The latter authors note the presence of eight and sixteen parasites in a single corpuscle; I have searched through several slides taken on different days and containing very heavy infections without finding more than six. Schwetz, Droeshaut & Peel (1933) described piroplasms from the blood of three Congo monkeys and identified them as *Babesia pitheci*; the dimensions of their forms resembled mine (2–6 μ). It is of interest that Kikuth (1927) also observed piroplasms in the blood of a *Cercopithecus* monkey (hitherto blood negative), 10 days after splenectomy; the resultant infection was accompanied by intense anaemia and caused the death of the animal. Fairbairn (1948) reported the presence of a piroplasm in East African monkeys, but this is quite different in morphology and was identified as *Entopolypoides macaci*. It is concluded that the Kenya parasite is *Babesia pitheci*.

SUMMARY

1. The following new species were encountered in this investigation:

Haemogregarina hamata in *Lacerta jacksoni*;
Hepatozoon minchini in *Crotaphopeltis degeni*;
Pirhaemocyton granosa in *Agama colonorum*;
Atoxoplasma argyae n.gen. n.sp. in *Argya rubiginosa*;
Spirochaeta harveyi in *Cercopithecus aethiops*.

2. Further information is given in regard to the following known species, including new host records:

Plasmodium pitmani in *Mabuia striata* and *M. maculilabris*;
Trypanosoma martini in *Mabuia maculilabris*;
Plasmodium vaughani in *Othyphantes reichenowi*;
Plasmodium relictum in *Othyphantes reichenowi* and *Passer iagoensis*;
Haemoproteus wenyoni in *Passer griseus*;
Leucocytozoon sp. in nine species of birds;
Hepatocystes epomophori in *Epomophorus labiatus*;
Hepatocystes kochi in *Cercopithecus*, etc.;
Babesia pitheci in *Cercopithecus mitis*.

3. Exo-erythrocytic schizogony is described in *Plasmodium pitmani* of the skink (from spleen and heart preparations).

I have to thank the members of the staff of the Coryndon Museum, Nairobi, for identification of birds, reptiles and mammals, and to Col. H. E. Shortt for encouragement and advice.

REFERENCES

ADIE, J. R. (1908). *Indian Med. Gaz.* **43**, 176.
ARAGÃO, H. DE B. (1933). *C.R. Soc. Biol., Paris*, **113**, 214.
BOUET, G. (1909). *C.R. Soc. Biol., Paris*, **66**, 609.
BRUMPT, E. & LAVIER, G. (1935). *Ann. Parasit. hum. comp.* **13**, 537.
FAIRBAIRN, H. (1948). *Ann. Trop. Med. Parasit.* **42**, 118.
FANTHAM, H. B. (1924). *S. Afr. J. Sci.* **21**, 435.
GARNHAM, P. C. C. (1947*a*). *E. Afr. Med. J.* **24**, 47.
GARNHAM, P. C. C. (1947*b*). *Trans. R. Soc. Trop. Med. Hyg.* **40**, 719.
GARNHAM, P. C. C. (1948). *Trans. R. Soc. Trop. Med. Hyg.* **41**, 601.
GIOVANNOLA, A. (1939). *Riv. Parassitol.* **3**, 221.
HERMAN, C. M. (1937). *Amer. J. Hyg.* **25**, 303.
HERMAN, C. M. (1941). *Amer. J. Hyg.* **34**c, 22.
HOARE, C. A. (1932). *Parasitology*, **24**, 210.

HUFF, C. G. (1942). *J. Infect. Dis.* **71**, 18.
KIKUTH, W. (1927). *Arch. Schiffs- u. Tropenhyg.* **31**, 37.
LAVERAN, A. & MESNIL, F. (1912). *Trypanosomes et Trypanosomiases.* Paris.
MANWELL, R. D. (1935). *Amer. J. Hyg.* **21**, 180.
MANWELL, R. D. (1939). *Riv. Malariol.* **18**, 76.
MANWELL, R. D., COULSTON, F., BINCKLEY, E. C. & JONES, V. P. (1945). *J. Infect. Dis.* **76**, 1.
MARTIN, G. (1907). *C.R. Soc. Biol., Paris*, **62**, 594.
MARULLAZ, M. (1913). *Bull. Soc. Path. Exot.* **6**, 323.
MINCHIN, E. A. (1910). *Rep. Sleep. Sickn. Comm. Roy. Soc.* **10**, 73.
NOBREGA, P. & REIS, J. (1942). *Arqu. Inst. Biol.* **13**, 21.
NOVY, G. & MACNEAL, W. Y. (1904). *Amer. Med.* **8**, 932.
NUTTALL, G. H. F. & GRAHAM-SMITH, G. S. (1908). *Parasitology*, **1**, 134.
PURCHASE, H. S. (1942). *Parasitology*, **34**, 278.
RAFFAELE, G. (1938). *Riv. Malariol.* **17** (i), 85.
RODHAIN, J. (1926). *Bull. Soc. Path. Exot.* **19**, 828.
RODHAIN, J., PONS, C., VANDEBRANDEN, F. & BEQUART, J. (1913). *Arch. Protistenk.* **29**, 259.
ROSS, P. H. (1905). *J. Hyg., Camb.*, **5**, 18.
SABIN, A. B. (1939). *Proc. Soc. Exp. Biol., N.Y.*, **41**, 75.
SCHWETZ, J. (1931*a*). *Ann. Parasit. hum. comp.* **9**, 193.
SCHWETZ, J. (1931*b*). *Ann. Parasit. hum. comp.* **9**, 303.
SCHWETZ, J. (1931*c*). *Ann. Parasit. hum. comp.* **9**, 311.
SCHWETZ, J. (1933*a*). *Ann. Parasit. hum. comp.* **11**, 287.
SCHWETZ, J. (1933*b*). *Zbl. f. Bakt.* **130**, 113.
SCHWETZ, J. (1935). *Riv. Malariol.* **14**, 214.
SCHWETZ, J. (1938). *Ann. Parasit. hum. comp.* **16**, 242.
SCHWETZ, J., DROESHAUT & PEEL (1933). *Zbl. f. Bakt.* **130**, 121.
SERGENT, ED. & SERGENT, ET. (1948). *Arch. Inst. Pasteur Algér.* **26**, 394.
SHORTT, H. E. (1917). *Indian J. Med. Res.* **4**, 402.
STILES, C. W. (1927). *Science*, **65**, 194.
THOMPSON, P. E. & HUFF, C. G. (1944). *J. Infect. Dis.* **74**, 48.
VAN DEN BERGHE, L. (1942). *Exploration du Parc National Albert et du Parc National de la Kagera.* 1. *Parasites du sang des Vertébrés.* Brussels.
WENYON, C. M. (1909). *Third Rep. Wellcome Trop. Res. Lab.* Khartoum.
WENYON, C. M. (1926). *Protozoology*, **2**. London.
WINGSTRAND, K. G. (1948). *K. svenska VetenskAkad. Handl.* III, **24**, no. 8.
WOLFSON, F. (1940). *Amer. J. Hyg.* **32***c*, 88.

EXPLANATION OF PLATES VII AND VIII

All figures are from camera lucida drawings (made by the author and completed by Mr W. Cooper) of smear preparations of blood or organs unless otherwise stated. Magnification × 2000.

PLATE VII

Figs. 1—9. *Plasmodium pitmani* (Figs. 1–5, blood smears; Figs. 6–9 heart or spleen smears).

Fig. 1. Oval form.
Fig. 2. Immature schizont.
Fig. 3. Double infection of R.B.C. with young trophozoite and schizont.
Fig. 4. Nearly mature schizont.
Fig. 5. Macrogametocyte.
Fig. 6. Young exo-erythrocytic schizont beside monocyte nucleus.
Fig. 7. Exo-erythrocytic schizont in monocyte.
Fig. 8. Vacuolated exo-erythrocytic schizont.
Fig. 9. Mature exo-erythrocytic schizont showing separation of merozoites.

Figs. 10–16. *Hepatozoon minchini* (Fig. 10, blood smear; Figs. 11–16, section of lung).

Fig. 10. Haemogregarine in erythrocyte.
Fig. 11. Early stage in schizogony.
Fig. 12–14. Later stages in schizogony.
Fig. 15. Mature schizont.
Fig. 16. Mature schizont with residual body.

Figs. 17–19. *Haemogregarina hamata.*

Fig. 17*a*. Uninfected erythrocyte.

Figs. 20–22. *Pirhaemocyton granosa.*

PLATE VIII

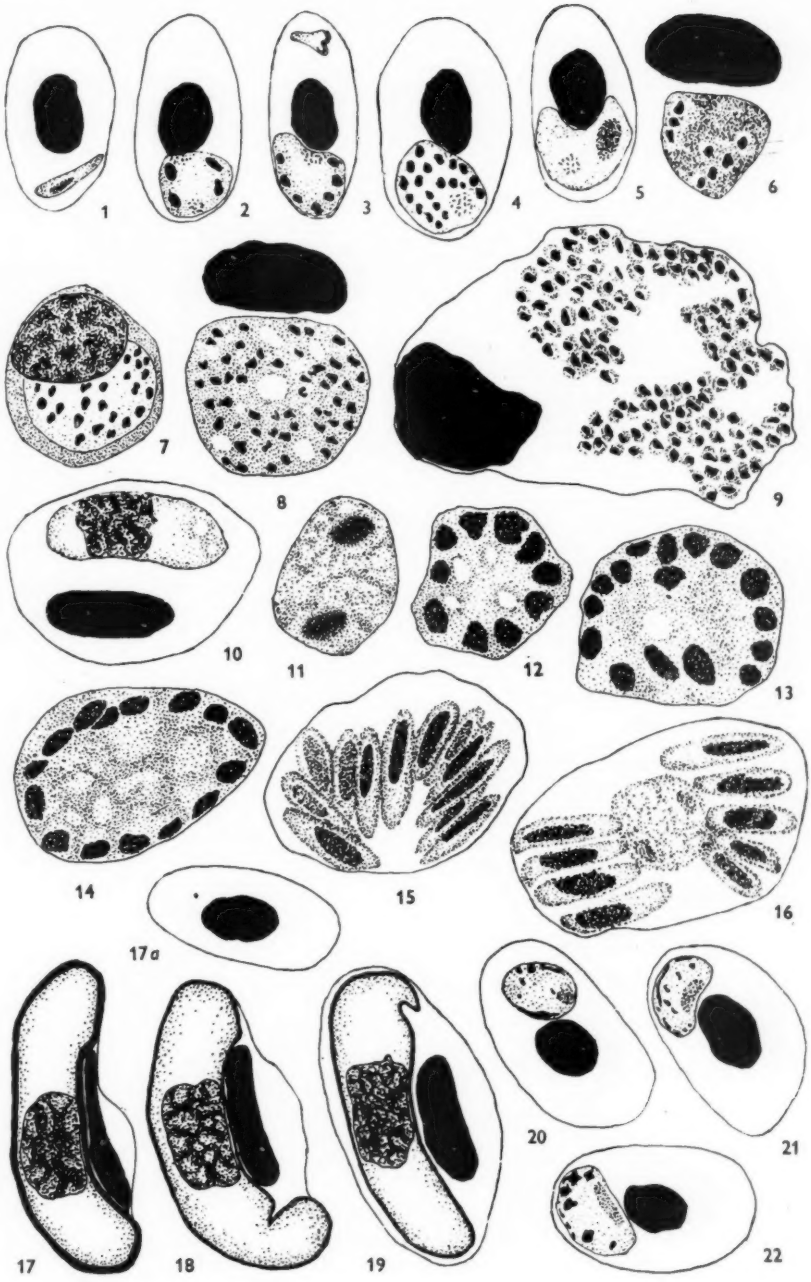
Figs. 23, 24. *Trypanosoma martini.*

Figs. 25–28. *Plasmodium vaughani.*

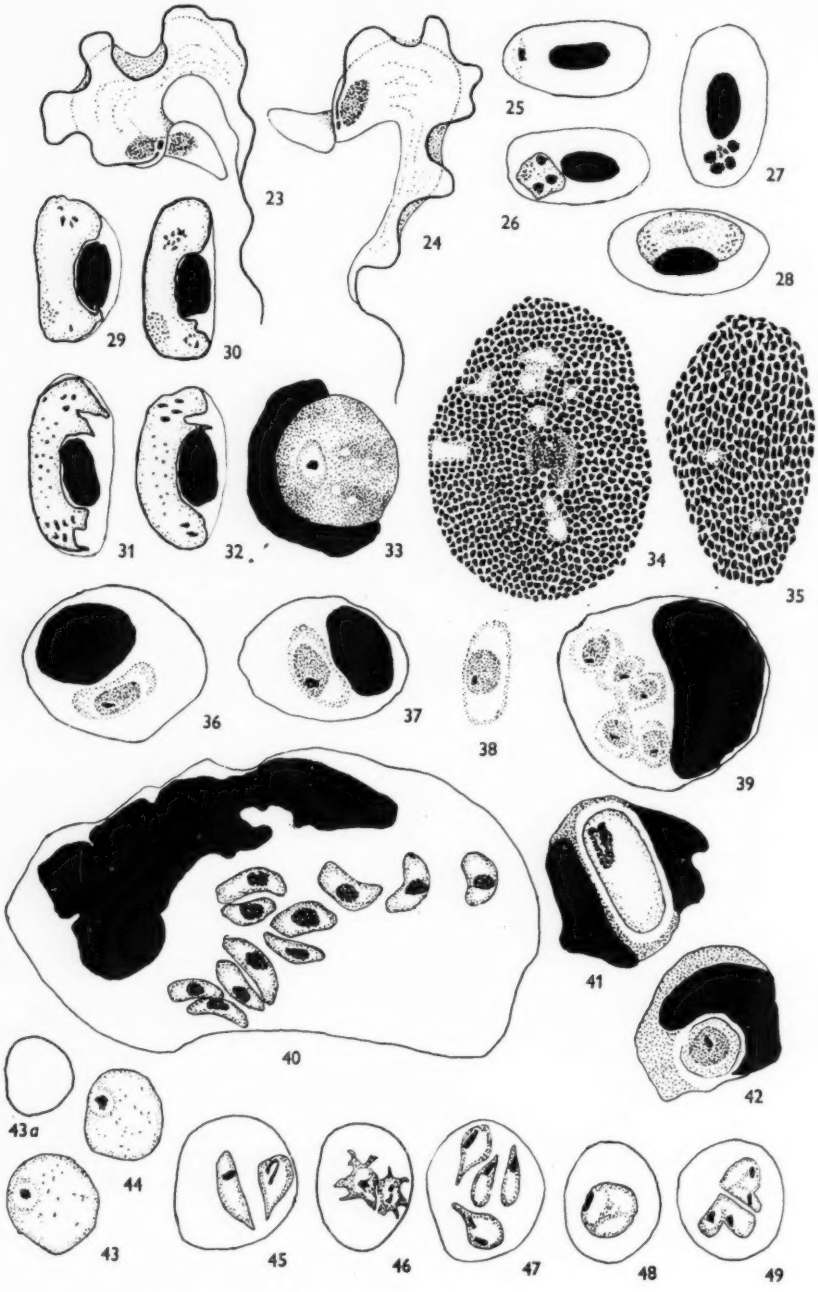
Fig. 25. Young band form.
Fig. 26. 'Nuttallia'-like schizont.
Fig. 27. Mature schizont with four merozoites.
Fig. 28. Gametocyte.

Figs. 29–32. *Haemoproteus wenyoni.*

Figs. 29, 30. Macrogametocytes.
Figs. 31, 32. Microgametocytes.



Figs. 1–22. ×2000



Figs. 23–49. ×2000

Figs. 33–35. *Leucocytozoon* (Fig. 33, blood smear; Fig. 34, spleen smear; Fig. 35, section of crow's spleen from material supplied by Dr Wingstrand).

Fig. 33. Macrogametocyte.
Fig. 34. Detached cytomere from ruptured megaloschizont packed with merozoites.
Fig. 35. Cytomere from megaloschizont of *L. sakharoffi*.

Figs. 36–39. *Atoxoplasma argyae* (Lung smears).

Figs. 36, 37. Intracellular forms.
Fig. 38. Extracellular parasite.
Fig. 39. Dividing forms in monocyte.

Figs. 40–42. Parasites resembling *Atoxoplasma*.

Fig. 40. *Toxoplasma gondi* from peritoneal fluid of infected mouse.
Fig. 41. *Hepatozoon adiei*. Blood smear of golden eagle from slide lent by C. A. Hoare.
Fig. 42. Young *Leucocytozoon* gametocyte, intracellular.

Figs. 43, 44. *Hepatocystes epomophori*.

Fig. 43*a*. Uninfected erythrocyte.
Figs. 43, 44. Macrogametocytes.

Figs. 45–49. *Babesia pitheci*.

Fig. 45. Oval and pear-shaped parasites.
Fig. 46. Amoeboid parasites.
Fig. 47. Four in a cell.
Fig. 48. Circular form.
Fig. 49. Forms in division.

(*MS. received for publication* 26. VIII. 1949.—Ed.)

THE EXCYSTATION OF *ENTAMOEBA HISTOLYTICA* WITHOUT BACTERIA IN MICROCULTURES

CHARLES W. REES, LUCY V. REARDON AND IDA LOUISE BARTGIS*

From the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland

WITH THE COLLABORATION OF DR LESLIE HELLERMAN, *Johns Hopkins University, School of Medicine*

(With 1 Figure in the Text)

Excystation of *Entamoeba histolytica* with bacteria has been described by a number of investigators including Yorke & Adams (1926), Dobell & Laidlaw (1926), Dobell (1928), and Cleveland & Sanders (1930*a*, *b*). These studies showed that cysts will excyst *in vitro* without previous passage through the upper digestive tract. In addition the production of four nuclei during encystation and morphological changes occurring during encystation and excystation, such as the production and dissolution of the glycogen body and the chromatoidal bodies, were well described. The materials in these bodies are probably reserves required for excystation but the chemical nature of the chromatoidal bodies is not well understood.

Excystation without bacteria has been reported by Cleveland & Sanders (1930*a*), Meleney, Frye, Leathers & Snyder (1940), Snyder & Meleney (1941), Rees, Reardon, Jones & Griffin (1947), and Rees, Reardon & Baernstein (1948), and some information has been obtained as to the cause of this phenomenon. The work of Meleney and associates indicates that a low oxygen content of the medium is necessary for excystation. The required reduction was produced by absorption of oxygen in alkalinized pyrogallol, aeration with nitrogen gas, and/or addition of reducing agents. A number of nutrient media were used but possible effects of organic compounds on excystation were not discussed. On the other hand, it was concluded that inorganic salts are necessary for excystation. The proportion of cysts excysting was low. Snyder & Meleney (1941) reported that 'in the presence of living bacteria the proportion of cysts excysting has usually been between 1 and 10%. In the absence of living bacteria the proportion is always less.'

Rees *et al.* (1947) obtained higher figures without bacteria in media containing complex organic materials. These results show that organic substances may be operative in excystation but furnish no information concerning specific organic compounds. The data cited above indicate the need for more information concerning the factors required for excystation. In the present work, fluids composed of measured amounts of inorganic compounds were compared with the same fluids enriched with organic compounds. A reducing agent was used with the objective of lowering the oxygen content of the medium.

* Technical expert.

MATERIALS AND METHODS

Cysts were obtained from the NRS strain of *E. histolytica* (Dobell, 1931) by supplying starch-starved cultures with rice powder as recommended by Dobell & Laidlaw (1926). As Dobell (1928) has pointed out that cysts do not hatch until mature, nuclear counts were made on samples of all harvests. Harvests showing less than 50% in the tetranucleate stage were discarded. The cysts were stained with iodine, although in some cases haematoxylin was used. Haematoxylin preparations, as well as unstained preparations in water, were examined for chromatoidal bodies as, according to Dobell, hatching does not occur if these bodies are still present in the cyst. In water the chromatoid bodies were not conspicuous; in the haematoxylin preparations they were seen in a small proportion of the cysts but only in the form of 'rice grains' (Faust, 1940). The cysts, therefore, were used without further reference to chromatoids. Cyst harvests were treated with distilled water and hydrochloric acid as suggested by Dobell for destruction of trophic amoebae. The acid served further to destroy many bacteria; those remaining were eliminated by the microisolation method of Rees (1942). Since microisolation of cysts from suspension containing rice and detritus is difficult, a method was developed for eliminating the bulk of these substances prior to microisolation. A heavy suspension of cysts and rice was placed in a small Petri dish, and after allowing time for the cysts to settle the dish was inverted. Many cysts remained in the dish and could be concentrated in the centre by adding Locke's solution and then gently rotating the dish. The method of Rees *et al.* (1947) was used for transferring the bacteria-free cysts from a Petri dish to microtubes constructed from capillary glass tubing.

The media were prepared, with designated exceptions, according to the formulae published by Anfinsen, Geiman, McKee, Ormsbee & Ball (1946) and employed by them for cultivating *Plasmodium knowlesi*. These formulae were used because they provided well-balanced solutions with measured ingredients that are known growth factors for many micro-organisms. A solution described as 'inorganic AG' was prepared by adding the following compounds to 1 l. of distilled water: NaCl, 5·83 g.; Na_2CO_3, 1·50 g.; KCl, 0·41 g.; Na_2HPO_4, 0·30 g.; $MgCl_2$, 0·20 g.; and $CaCl_2$, 0·067 g. Precipitates were dissolved by passing carbon dioxide into the fluid after it was autoclaved, until a pH at or near 7·4 was attained. The fluid, therefore, contained bicarbonates in addition to its other salts, and it also contained dissolved carbon dioxide. A fluid was prepared from the same ingredients (except carbonate) as 'inorganic AG' without dissolving the precipitates and was designated as 'phosphate'; enough buffer remained to produce a pH near 7·4. 'Inorganic AG glucose' and 'phosphate glucose' contained 3·0 g./l. of glucose added to the respective inorganic solutions. 'Complete AG' was prepared by adding to 'inorganic AG' the following: glucose, 3·0 mg./ml.; cocarboxylase, B vitamins, glucosamine, folic acid, and cholesterol, 0·5–1·0 γ/ml.; and vitamin C 5 γ/ml. 'Complete AG Elamine' contained Elamine, an acid hydrolysate of casein which, according to the manufacturers, has 13% nitrogen with 75% in α-amino-acids. Cysteine hydrochloride in the amount of 0·025 M was added to all of the above solutions except one in which 0·025 M-glutathione was used instead. The reducing agents were neutralized, without premature oxidation, by the use of a Luer syringe with a vaseline-coated plunger as described by Rees *et al.* (1948). After transferring the cysts to the respective solutions in microtubes the latter were sealed in a flame and incubated at 37° C. The readings were made after 8–10 hr. incubation by direct microscopic observation of the microtube immersed in water, using the 16 mm. or the 8 mm. objective.

EXPERIMENTAL DATA

The experimental data in Table 1 and illustrated in Fig. 1 constitute only a small number of the results obtained; for example, the data on media without reducing agents are not summarized as they are in agreement with those reported by Meleney and associates and confirmed by Rees *et al.* (1948) in showing that practically no excystation occurred without reduction of the oxygen content of the medium. The data in Table 1 are comparative results on excystation from six media per day with one cyst harvest per day. This practice was necessary because some harvests gave surprisingly low percentages of excystation and other harvests gave high percentages, and these differences in apparent viability were not correlated with figures obtained from the nuclear counts. The data in Table 1 and Fig. 1* show an increase in the percentages of microtubes in which fair percentages of excystation occurred, and a decrease in the percentages in which no excystation occurred, as the inorganic media were enriched by the addition of glucose or glucose plus vitamins and other compounds. The statisticians reported that: 'The probability is less than 1% that differences in percentages of excystation as large as those shown in the over all data could be due to chance.' However, when small differences such as those shown between

Table 1. *Comparative percentages of excystation of* Entamoeba histolytica *without bacteria from indicated media in microcultures containing ten cysts per culture*

No.	Medium	No. of micro-cultures	Percentage of microcultures with indicated number of amoebae									Total cysts	Total amoebae	Average excystation (%)
			0	1	2	3	4	5	6	7	8			
1	Phosphate cysteine	95	62·1	25·2	9·5	2·1	1·0	—	—	—	—	950	52	5·5
2	Inorganic AG cysteine	106	48·6	31·8	15·9	3·7	—	—	—	—	—	1060	80	7·5
3	Phosphate glucose cysteine	106	41·5	29·2	17·0	9·4	2·8	—	—	—	—	1060	109	10·3
4	Inorganic AG glucose cysteine	106	26·9	30·8	21·1	12·5	7·7	1·0	—	—	—	1060	152	14·3
5	Complete AG Elamine cysteine	106	16·2	31·4	22·9	12·4	7·6	7·6	1·0	—	—	1060	198	18·7
6	Complete AG Elamine glutathione	90	10·0	28·9	28·9	18·8	4·4	6·7	1·1	—	1·1	900	189	21·0
7	Complete AG cysteine	29*	—	—	20·7	20·7	58·5	—	—	—	—	725	215	29·6

* These experiments were conducted with twenty-five cysts per microtube.

* The authors wish to express their thanks to J. E. Lieberman and M. A. Schneidermann of the statistical staff of the National Institutes of Health and the National Cancer Institute for their help in the design of this phase of the experiments and for statistical interpretation and evaluation of the data.

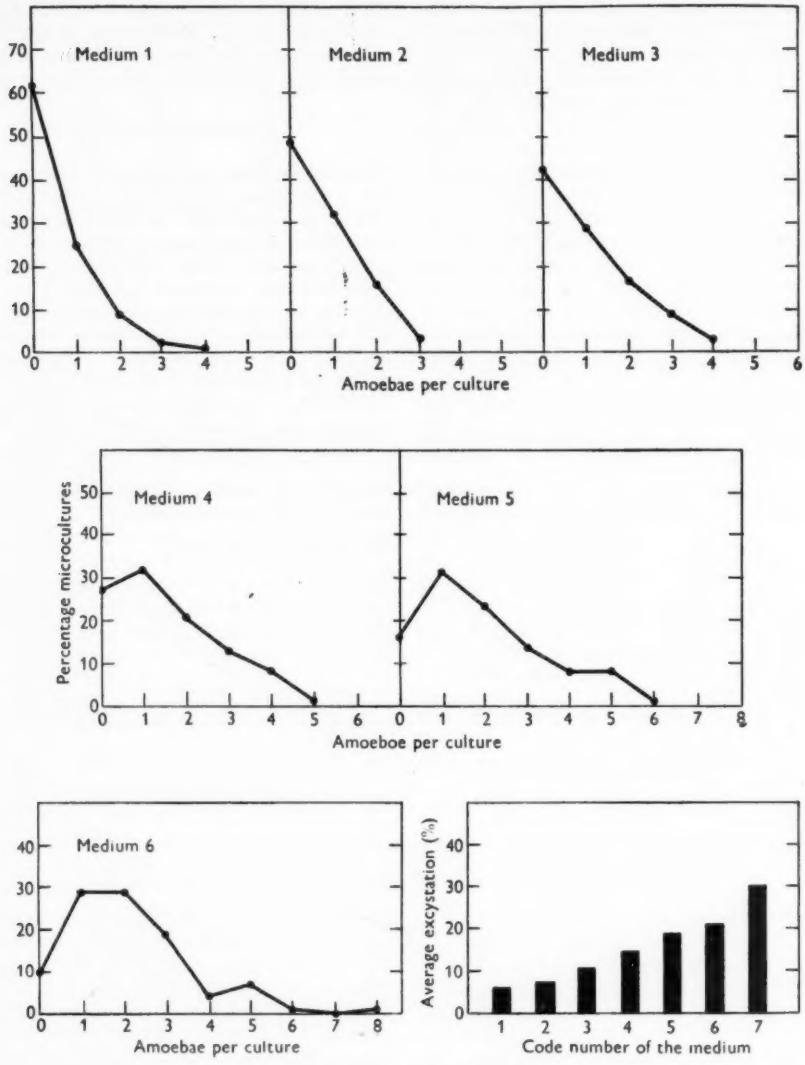


Fig. 1. Effects of organic compounds added to inorganic solutions plus a reducing agent on percentages of excystation of *Entamoeba histolytica* without bacteria in microcultures. Media: (1) inorganic compounds without bicarbonates, plus cysteine; (2) inorganic compounds including bicarbonates, plus cysteine; (3) same as (1) plus glucose; (4) same as (2) plus glucose; (5) same as (1) plus glucose, B vitamins, vitamin C, cocarboxylase, purines, pyrimidines, folic acid, glucosamine, cholesterol, and amino-acids; (6) same as (5) with glutathione substituted for cysteine; (7) same as (5) without amino-acids.

inorganic AG cysteine and phosphate cysteine, complete AG Elamine cysteine and complete AG Elamine glutathione were analysed, the probability that the differences may have been due to chance was found greater than 1%. The statisticians reported that long series of experiments will be necessary to ascertain whether the differences in question are significant. The data from the seventh medium, complete AG cysteine, show that high percentages of excystation may be obtained in the absence of

amino-acids. The differences shown between this medium and complete AG Elamine cysteine are probably not significant.

In view of the recent success of Phillips (1950) in cultivating *E. histolytica* without bacteria in the presence of *Trypanosoma cruzi*, some experiments were conducted with the objective of obtaining excystation with *T. cruzi*. In three experiments of ten microtubes, each with ten cysts per tube, high percentages of excystation were obtained. Exact figures on excystation were not obtained, since metacystic development and division occurred in the presence of *T. cruzi* and the number of metacystic amoebae could not therefore be counted. It may be necessary to conduct experiments with one cyst per culture to obtain such figures.

DISCUSSION

The percentages of excystation of *Entamoeba histolytica* in the absence of bacteria obtained in the present studies were higher than those recorded in any previous work, except that of Rees *et al.* (1948). The fairly high percentage of excystation and the method of direct examination of microtubes used in this work now make possible studies, which were not previously feasible, on the comparative effects of various media. Thus, while the failure of other workers to record comparative figures on excystation in various media may be attributable both to low rates of excystation and to difficulties in counting cysts and amoebae in their preparations, the microtube technique obviates such difficulties. It may thus be possible by the elimination method, to ascertain exactly which ingredients are necessary for the cysts.

Snyder & Meleney (1941) furnished little information on the roles of particular inorganic compounds since they reported excystation in modifications of Locke's solution and stated that the phenomenon did not occur in physiological sodium chloride nor in Ringer's solution. The present work has also supplied few data on this question, although there are indications that bicarbonates and possibly dissolved carbon dioxide may have beneficial effects. The only factors that appear to have been considered in previous work on preparation of fluids for cultural studies on the protozoa were pointed out as long ago as 1924 by Bayliss. In discussing the relative merits of Ringer and Locke's solutions for physiological studies on the heart beat and on micro-organisms, Bayliss stressed the need for isotonicity with body fluids, the use of salts, including bicarbonates, that occur in plasma and sea water, and dissolved carbon dioxide. Recent studies in enzymology have furnished additional evidence of the necessity of providing certain ions. These studies show that inorganic ions are required for enzymatic reactions in cellular metabolism. Pertinent references to literature dealing with ionic catalysis were cited by Sumner & Somers (1947); the work of Euler, Nilsson & Auhagen (1931), for example, showed that the magnesium ion is required for the enzymatic degradation of glucose. Important leads may also be found in the literature on bacteriology, which has been reviewed by Porter (1947) and Stephenson (1949). Thus the work of Quastel & Webley (1942) showed the need for ions of magnesium and potassium in the utilization of vitamin B_1 by propionic acid bacteria. The observation of Snyder & Meleney on the failure of cysts to excyst in Ringer's or physiological NaCl solutions while they did so in Locke's solution may therefore, if clarified, be of fundamental importance. The demonstration that *E. histolytica* cysts are dependent on certain ions might also lead to more intimate knowledge of the mechanism of excystation.

Although the conclusion of Meleney and associates that a lowered oxygen content of the medium is prerequisite for excystation has been confirmed, the question whether complete or only partial removal of the oxygen is necessary has not been answered. For the experiments described above, the concentration of 0·025M-cysteine was chosen arbitrarily to avoid complications that would have arisen in statistical analysis if the amount used was not constant. In work not reported above, cysteine was used in amounts of from 0·0125 to 0·05M with fair percentages of excystation in all concentrations. In some experiments with incubation of the cultures in McIntosh and Fildes's jars the percentages of excystation were not higher than in sealed cultures. However, even in the atmosphere of pure hydrogen of the McIntosh and Fildes's jar some dissolved oxygen may have been retained in the microtubes because of the limited boundary through which diffusion could occur. While it appears that reduction of the O_2 tension is necessary for excystation, the limits of O_2 tolerance of cysts have not been established.

According to Dobell (1927) 'the amount of reserve material in a cyst, determines not only the time for which it can survive, but also the rapidity with which it hatches'. The work reported here suggests that organic nutrients in particular media may have supplemented scanty reserve material in some of the cysts and that such cysts hatched in the presence of organic compounds but failed to hatch in the inorganic media. While proof of this hypothesis must await the demonstration of utilization of organic compounds by the amoeba within the cyst, the results thus far raise the question why metacystic amoebae failed to divide in the presence of such compounds. In many observations not a single case of division was noted in the absence of bacteria or *Trypanosoma cruzi*.

The data in the present paper show that higher

percentages of excystation were obtained in the presence than in the absence of glucose, and that the highest percentages occurred in the presence of inorganic salts, glucose, B vitamins, and other growth factors. Further work is necessary to ascertain which of the organic compounds are effective and which may be superfluous or even detrimental.

SUMMARY

1. Formulae developed by Anfinsen *et al.* (1946) for a medium used in the cultivation of *Plasmodium knowlesi* were used for media in which excystation of *Entamoeba histolytica* without bacteria was investigated.

2. The following media were used: (i) an inorganic fluid containing carbon dioxide and chlorides, phosphates, and bicarbonates, of sodium, potassium, magnesium, and calcium; (ii) an inorganic fluid of chlorides and phosphates of the above metals without bicarbonates; (iii) the same respective fluids plus glucose, and (iv) the same plus enrichments with B vitamins, cocarboxylase, vitamin C, purines, pyrimidines, folic acid, glucosamine, cholesterol, and amino-acids. The oxygen content of all media was lowered either by cysteine or glutathione.

3. Some excystation occurred in all media, the percentages were lowest in the inorganic fluid without bicarbonates, highest in fluid with bicarbonates plus all of the listed organic compounds, and intermediate in the inorganic bicarbonate fluid plus glucose. Amino-acids were not required for good percentages of excystation.

4. Excystation occurred when cysts were isolated in medium with *Trypanosoma cruzi*.

5. The data show that organic compounds in the medium are necessary for high percentages of excystation.

REFERENCES

Anfinsen, C. B., Geiman, Q. M., McKee, R. W., Ormsbee, R. A. & Ball, E. G. (1946). Studies on malarial parasites. VIII. Factors affecting the growth of *Plasmodium knowlesi in vitro*. *J. Exp. Med.* **84**, 607.

Bayliss, W. M. (1924). *Principles of General Physiology*. London: Longmans, Green and Co.

Cleveland, L. R. & Sanders, E. P. (1930*a*). The production of bacteria-free amoebic abscesses in the liver of cats and observations on the amoebae in various media with and without bacteria. *Science*, **72**, 149.

Cleveland, L. R. & Sanders, E. P. (1930*b*). Encystation, multiple fission without encystment, excystation, metacystic development, and variation in a pure line and nine strains of *Entamoeba histolytica*. *Arch. Protistenk.* **70**, 223.

Dobell, C. (1927). Further observations and experiments on the cultivation of *Entamoeba histolytica* from cysts. *Parasitology*, **19**, 288.

Dobell, C. (1928). Researches on the intestinal protozoa of monkeys and man. I. General introduction. II. Description of the whole life-history of *Entamoeba histolytica* in cultures. *Parasitology*, **20**, 357.

Dobell, C. (1931). IV. An experimental study of the histolytica-like species of *Entamoeba* living naturally in macaques. *Parasitology*, **23**, 1.

Dobell, C. & Laidlaw, P. P. (1926). On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. *Parasitology*, **18**, 283.

Euler, H., von Nilsson, R. & Auhagen, E. (1931). Ueber die Function des Magnesiums beim enzymatischen Kohlenhydratbau. *Hoppe-Seyl. Z.* **200**, 2.

Faust, E. C. (1940). The carrier races of *Endamoeba histolytica* in a New Orleans Children's Institution. *J. Parasit.* Supp. **26**, 21.

Meleney, H. E., Frye, W. W., Leathers, W. S. & Snyder, T. L. (1940). The sterilization of the cysts of *Endamoeba histolytica*, with preliminary observations on subsequent excystation. *Proc. 3rd Int. Congr. Microbiol.* p. 410.

Phillips, B. P. (1950). The cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*. *Science*, **111**, 8.

Porter, J. R. (1947). *Bacterial Chemistry and Physiology*. London: John Wiley and Sons.

Quastel, J. H. & Webley, D. M. (1942). Vitamin B_1 and bacterial oxidations. 2. The effects of magnesium, potassium, and hexose diphosphate ions. *Biochem. J.* **36**, 8.

Rees, C. W. (1942). The construction of a micromanipulator for the isolation of protozoa. *Amer. J. Trop. Med.* **22**, 487.

Rees, C. W., Reardon, L. V. & Baernstein, H. D. (1948). A biochemical approach to the problem of cultivating *Endamoeba histolytica* without bacteria. *J. Parasitol.* **34** (Sec. 2), 11.

Rees, C. W., Reardon, L. V., Jones, F. E. & Griffin, A. M. (1947). Observations on the excystation of *Endamoeba histolytica*. *J. Parasit.* **33**, 385.

Snyder, T. L. & Meleney, H. E. (1941). The excystation of *Endamoeba histolytica* in bacteriologically sterile media. *Amer. J. Trop. Med.* **21**, 63.

Stephenson, M. (1949). *Bacterial Metabolism*, 3rd ed. London: Longmans, Green and Co.

Sumner, J. B. & Somers, G. F. (1947). *Chemistry and Methods of Enzymes*. New York: Academic Press Inc.

Yorke, W. & Adams, A. R. D. (1926). Observations on *Entamoeba histolytica*. I. Development of cysts, excystation, and development of excysted amoebae *in vitro*. *Ann. Trop. Med. Parasit.* **20**, 279.

(*MS. received for publication* ii. x. 1949.—Ed.)

AN EXPERIMENTAL STUDY OF *ENTAMOEBA MURIS* (GRASSI, 1879);* ITS MORPHOLOGY, AFFINITIES AND HOST-PARASITE RELATIONSHIP

By R. A. NEAL

From the Wellcome Laboratories of Tropical Medicine, 183–193 Euston Road, London, N.W. 1

(With 23 Figures in the Text)

CONTENTS

	PAGE
I. Introduction	343
II. Morphology and life history of *Entamoeba muris*	344
(1) Review of previous work	344
(2) Material and methods	345
(3) Description of the amoebae	345
(4) Nuclear division	347
(5) Description of the cysts	349
(6) Discussion of the morphological characters of *Entamoeba muris*	353
III. Experimental infections with *Entamoeba muris*	354
(1) Material and methods	355
(2) Infection of mice with a rat strain of *Entamoeba muris*	356
(3) Infection of rats with a mouse strain of *Entamoeba muris*	356
(4) Infection of rats and mice with a hamster strain of '*Entamoeba muris*'	357
(5) Observations on infections with *Entamoeba muris*	357
IV. Attempts to infect rats and mice with *Entamoeba coli*	358
(1) Materials and methods	358
(2) Attempts to infect rats with *Entamoeba coli* from man	359
(3) Attempts to infect rats and mice with cultured strains of *Entamoeba coli*	359
V. Incidence and geographical distribution of *Entamoeba muris*	360
(1) Incidence	360
(2) Geographical distribution	361
VI. Host-restriction of *Entamoeba muris*	361
VII. The classification of *Entamoeba muris*	361
VIII. Summary	363
IX. References	363

I. INTRODUCTION

The amoebae, which inhabit the alimentary canal of rats and mice, have been studied and classified into various genera and species by several workers, the last paper dealing with the taxonomy of these protozoa having been published by Kessel in 1924. The interest in the morphology and biology of these amoebae has been recently stimulated by the use of rats as experimental hosts for infection with *Entamoeba histolytica* (Jones, 1946; Goodwin, Hoare & Sharp, 1948), and the use of this technique for screening new drugs for possible activity against human amoebiasis caused by *E. histolytica* (Goodson, Goodwin, Gorvin, Goss, Kirby, Lock, Neal, Sharp & Solomon, 1948*a*,*b*).

Since the presence of *E. muris* introduces a complicating factor in the experimental infections (Neal, 1949), it is essential to differentiate natural infections with this species from experimental infections with *E. histolytica*. However, an examination of the literature on the murine amoebae revealed some confusion regarding the structure, bionomics, and classification, on account of which it was decided to undertake a revision of *E. muris*.

In the following description, the name *E. muris* is used to describe the amoebae occurring in rats and mice. The term 'strain' is used to denote batches of cysts from a particular animal or culture, whereas 'race' is used to describe various strains of the same species of amoebae which differ only in the size of their ripe cysts.

* Thesis approved by the University of London for the award of the degree of Ph.D.

II. MORPHOLOGY AND LIFE HISTORY OF *ENTAMOEBA MURIS*

(1) *Review of previous work*

The presence of amoebae parasitic in rats and mice was first noted by Grassi (1879). In 1882, he gave a further description of these protozoa in which he stated that the amoebae were similar to *E. coli* of man, but were smaller than the human species.

Wenyon (1907) described the amoebae and cysts found in the mouse. He concluded that they resembled *E. coli* very closely and stated that 'it is difficult to avoid the conclusion that they are identical'. Some of his interpretations are not recognized; thus a process of autogamy was described, and the chromatoid bodies were regarded as waste food products and extra-nuclear chromatin, nor did he realize the importance of the nuclear structure as a specific character.

Brug (1919) published observations upon the structure of the cysts and amoebae found in rats. He also considered the amoebae of rats and mice to be indistinguishable from *E. coli* of man.

The above-named workers placed the amoebae of rats and mice in one species, *E. muris*. Rudovsky (1921), however, described morphological differences between the rat amoeba, which he named *E. muris decumani*, the amoeba of the mouse *E. muris*, and *E. coli*. He based these species upon differences of nuclear structure. The nucleus of *E. coli* was described as possessing a coarse peripheral layer of chromatin, a large karyosome, and chromatin granules between the nuclear membrane and the karyosome; *E. muris* was similar, but the chromatin granules were absent; while *E. muris decumani* possessed a thin peripheral layer of chromatin, with a small karyosome and small chromatin granules between the nuclear membrane and the karyosome

Kessel (1924) transferred *E. muris* and *E. muris decumani* to the genus *Councilmania* which, according to him, occurred in both rats and mice, and described a third species, *Endamoeba ratti*, found only in rats. The *Councilmania* species resembled one another in possessing pronounced ectoplasmic pseudopodia, a dispersed karyosome, and in the method of excystation, which was said to consist of a process of budding from a deeply stained region called the chromophile ridge. The nucleus of *C. muris* had very little chromatin upon the nuclear membrane and the karyosome consisted of a number of dispersed granules which were central or eccentric in position. The nuclear membrane of *C. decumani*, on the other hand, was lined with a thicker layer of chromatin, and the karyosome granules were less dispersed than those of *C. muris*. The trophic amoebae of *C. muris* and *C. decumani* measured 19 μ in diameter.

C. muris was divided into three races, the cysts of which measured 14·5, 15·7 and 17·7 μ in diameter, while the racial sizes of the cysts of *C. decumani* were 14·0, 15·5 and 17·0 μ. The number of nuclei possessed by the mature cysts of *C. muris* was 8, while the corresponding number for *C. decumani* was 8–16.

Kessel distinguished *Endamoeba ratti* from the *Councilmania* species by the structure of the pseudopodia and the nucleus. *Endamoeba ratti* moved by the protrusion of pseudopodia composed only of endoplasm, the peripheral chromatin of the nucleus was of a thickness between that of *Councilmania muris* and *C. decumani*, and the karyosome was represented by a single granule eccentric in position. The ripe cysts measured 14·5 μ in diameter and contained eight nuclei.

The division of the nucleus was described from cystic nuclear divisions. The validity of *C. muris* and *C. decumani* was upheld by cross-infection experiments from one host to the other, during which the morphological differences were retained.

Chiang (1925*a*, *b*) gave a short description of *Entamoeba muris* from laboratory rats, and discovered another amoeba parasitic in rats, which was morphologically identical to *Endolimax nana*. Since he was unable to produce in rats an experimental infection of *E. nana* of human origin, he concluded that it was biologically distinct from the human species. The rat amoeba, the mature cysts of which were quadrinucleate, he named *E. ratti*.

A short note upon the morphology of *Entamoeba muris* was published by Wang & Nie (1934), and Wenrich (1941*a*) gave a description of the food ingested by amoebae of *E. muris*. He showed that the amoebae ingested diverse objects of animal and vegetable origin, and that some amoebae (at least for a short time) engulf one type of food particle only, while others may ingest a variety of objects.

In 1929, Epstein & Matevosjan separated the amoebae of rats into four 'types' based upon the size and the nuclear structure of the amoebae. This classification was described in greater detail, in a posthumous monograph by Epstein (1941). In the latter publication these types were given specific status and a fifth species was added. From the descriptions, three of these species, *E. minuta* var. *ratti*, *E. tenuis* var. *ratti*, and *E. histolytica* var. *muris*, were forms of *E. histolytica* and will not be considered here. As this work is written in Russian the description of the remaining forms will be summarized in greater detail.

(*a*) *E. muris*. The amoeba measures 25–30 μ and forms homogeneous pseudopodia, the ectoplasm is clearly differentiated from the endoplasm. The nucleus which measures 6–8 μ in diameter is characterized by a small eccentric karyosome, the remainder of the nucleus contains a finely granular network. Epstein also describes problematical

structures which are termed 'spheres'. These spherical structures are said to occur in the majority of nuclei and may be small, hardly exceeding the karyosome in size, or they may attain enormous dimensions, up to half the size of the nucleus. Eight-nucleate and binucleate cysts of the *E. coli* and *E. muris* type were seen.

(*b*) *E. coli* var. *ratti*. The trophic amoebae measure 25 μ and resemble *E. coli* in the absence of differentiation of ectoplasm and endoplasm, sluggish movement, fissure-like vacuoles, and a centrally placed, slightly elongated karyosome.

Recently, Fulton & Joyner (1948) published brief descriptions of amoebae which occurred in various laboratory rodents, including white rats.

Natural infections with *E. histolytica* in rats have been described (see Neal, 1948), but have not been reported from mice.

(2) *Material and methods*

The present description is based on the examination of permanent and fresh smears prepared from many laboratory and a few wild rats (*Rattus norvegicus* and *R. rattus*), and from laboratory mice (*Mus musculus*). In addition, four strains of *Entamoeba muris* were passed *in vivo* and examined cytologically. Two of these strains were isolated from laboratory rats, one from a wild rat, and one from a laboratory mouse. All these strains were identical in morphology.

The movement of amoebae was observed by preparing a smear with normal saline from caecal contents and maintaining it at a temperature of 37° C. by means of a warm stage. Evaporation was prevented by sealing the cover-slip with Czokor's wax.

Permanent slides for the morphological study of amoebae and cysts were prepared from the caecal contents of freshly killed rats and mice; good preparations of cysts were also made from infected faeces. The caecal contents were smeared direct upon cover-slips. Wenrich (1940) in his study of nuclear division diluted the material with saline before fixing, but it was found that this process was unnecessary and tended to increase the degree of vacuolation of the endoplasm of trophic amoebae.

Most of the cytological fixatives used gave good results with *E. muris*, although Schaudinn's fluid with 5% acetic acid added, and Bouin's fluid, were usually employed. Carnoy's fluid also gave well-fixed preparations. Zenker's fixative (original formula) was found to fix nuclear details well, but the cytoplasm and its inclusions were not sharply defined. All fixatives were used at room temperature, about 17–20° C.

Smears were stained with Heidenhain's haematoxylin with or without counterstaining in eosin, or with Dobell's phosphotungstic haematoxylin (1942). Cooper's modification of Dobell's original method was also used and was carried out as follows: the fixed smear was mordanted in 2% phosphotungstic acid for 2–3 hr., and then transferred without washing to 0·5% haematoxylin in which it was stained overnight at 37° C. The phosphotungstic haematoxylin stain gave especially good results. Feulgen's 'nucleal reaction' was tried but gave unsatisfactory results which are described in greater detail later (p. 347). Unsatisfactory preparations were also obtained with one sample of chlorazol black.

Temporary preparations were tinted with Lugol's iodine solution, which revealed the presence of glycogen. This was confirmed by staining with Best's carmine stain after fixation with Carnoy's fluid.

Attempts were made to cultivate *E. muris in vitro* using Dobell & Laidlaw's (1926) and other media, but these experiments were not successful. Kuwabara (1931) and Frye & Meleney (1932) reported the successful cultivation of *E. muris* and the media employed by these authors were also tried without success.

(3) *Description of the amoebae*

Size. Rounded amoebae when alive measure from 12 to 30 μ in diameter, but when extended during active movement larger measurements may be recorded. Wenyon (1907) records that amoebae may measure 30–40 μ.

Pseudopodia and movement. In a fresh smear made from an infected rat or mouse, sealed to prevent evaporation and kept at 37° C., practically all amoebae are seen protruding ectoplasmic pseudopodia. A small number of amoebae are observed to move very sluggishly, without forming pronounced ectoplasmic pseudopodia, but this is not the normal mode of progression. Upon cooling the amoebae retract their pseudopodia, and very quickly cease moving. Kessel (1924) gives a more detailed account of the movement and pseudopodia of amoebae.

Cytoplasm. The ectoplasm forms a layer of variable thickness over the whole surface of the amoeba and is usually distinct from the granular endoplasm (Figs. 1, 2 and 6), but in some cases it merges imperceptibly with the endoplasm (Fig. 3). When fixed and stained, the ectoplasm appears homogeneous and finely granular, whereas the endoplasm is coarsely granular and contains food vacuoles and the nucleus.

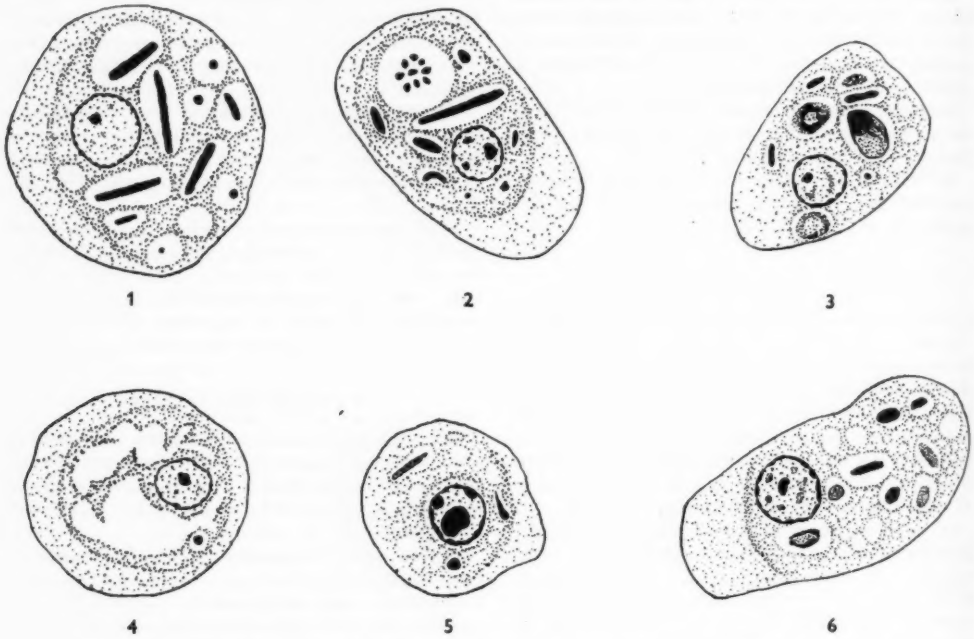
As previously shown by Wenrich (1941*a*), food vacuoles contain bacteria or protozoa (commonly trichomonads), but any other objects may be ingested, for instance red blood corpuscles, if they are available. Amoebae are occasionally parasitized by *Sphaerita* (Fig. 2).

Degenerate individuals are characterized by

possessing a large number of empty vacuoles in the endoplasm, and are common on slides which have partially dried before fixation. The endoplasm becomes vacuolated and disintegrates before the ectoplasm. If a thick ectoplasmic layer is present, it is possible to observe amoebae which in optical section, present the appearance of a nucleus surrounded by a ring of ectoplasm, the endoplasm having almost entirely disappeared (Fig. 4). Eventually the ectoplasm also disintegrates leaving the nucleus in the fluid medium. This artefact is often present if the caecal contents are diluted with saline before fixation.

Nucleus. The nucleus is visible in the living animal, although its conspicuousness varies according to the nature of the vacuoles present. The peripheral layer of chromatin can be seen as a beaded ring, but the karyosome is only faintly visible, often being entirely invisible. However, by colouring a fresh preparation with iodine solution the characteristic structure of the nucleus may be observed.

The peripheral layer of chromatin in normal healthy amoebae is regularly arranged in blocks of approximately equal size lining the nuclear membrane. The thickness and arrangement of the chromatin in nuclei of individual amoebae varies slightly (Figs. 1–3 and 6). In degenerate specimens, the peripheral layer of chromatin may be almost absent (Fig. 4) or the chromatin may be irregularly lumped upon the nuclear membrane (Fig. 5).



Figs. 1–6. Trophic amoebae of *Entamoeba muris* from rats and mice. × 2000 (2 mm. = 1 μ).

Details of the method of preparation of figures in Figs. 1–23 are abbreviated as follows: S = fixed in Schaudinn's fluid, B = fixed in Bouin's fixative, H = stained with Heidenhain's haematoxylin, P = stained with phosphotungstic haematoxylin. See text for further explanation of figures.

Fig. 1. Amoebae from rat with ectoplasm distinct from endoplasm which contains ingested bacteria. S, H.
Fig. 2. Amoeba from rat, with the nucleus containing granules of chromatin. Amoeba parasitized by *Sphaerita*. S, H.
Fig. 3. Amoeba from mouse, with ectoplasm not distinct from endoplasm. S, H.
Fig. 4. Degenerate amoeba from rat, with endoplasm almost entirely vacuolate whilst the ectoplasm is intact. Peripheral chromatin of nucleus almost absent. S, H.
Fig. 5. Abnormal amoeba from rat, with the karyosome very large, and peripheral chromatin irregularly lumped. S, H.
Fig. 6. Amoeba from mouse, with the chromatin granules of the nucleus slightly flocculated. B, P.

Between the karyosome and the peripheral layer a few granules of chromatin are usually present, and may be diffusely arranged throughout the nucleus (Figs. 3, 6) or these granules may be more definite, approaching the karyosome in size (Fig. 2). I was unable to discover any structures resembling the spheres described by Epstein (1941).

The karyosome of *Entamoeba muris* is characteristically eccentric in position; it was centrally placed in less than 5% of amoebae examined. It is a compact irregularly rounded structure, which measures from 0·25 to 1·50 μ in diameter, more usually from 0·50 to 0·75 μ in diameter, and varies in position from extreme eccentricity, almost touching the nuclear membrane, to a central position in the nucleus (Figs. 1–3 and 6). In abnormal amoebae, the karyosome may reach an enormous size (Fig. 5).

The nucleus is more resistant to disintegration than the cytoplasm, and it was observed intact in both fresh and permanent preparations after the cytoplasm of the amoeba had disintegrated.

Since Epstein & Matevosjan (1929) and Epstein (1941) distinguished two species of amoebae in rodents, *E. muris* and *E. coli* var. *ratti*, which differ chiefly in the eccentricity of the karyosome of the nucleus, the degree of eccentricity was examined statistically.

The distance between the karyosome and the peripheral chromatin of nuclei was measured in a large number of amoebae (500) of *E. coli* (MC culture) and of *E. muris* (natural infection of a rat and of a mouse), and the results compared by plotting the frequency distribution of these measurements. It was found that the graphs were not significantly different, but a greater number of nuclei of amoebae with extremely eccentric karyosomes were found in *E. muris* than in *E. coli*. This feature was not, however, sufficiently marked to be a distinguishing character between the two species.

(4) *Nuclear division*

Descriptions of nuclear division of *E. muris* have been reported by Kessel (1924), who described those occurring in cystic nuclei, and by Wenrich (1940) who described the division of nuclei of trophic amoebae.

According to Kessel, the granules of the karyosome become somewhat more dispersed than usual, the granules on the nuclear membrane disappear and a darkly staining region appears around the granules arranged in the middle of the nucleus. An intradesmose appears between two of these granules; these separate and finally take their positions at the ends of the spindle. Between these granules a spindle appears, and the chromosomes arrange themselves upon this structure. He does not describe the origin of the chromosomes but says: 'the granules of the dispersed karyosome and the dark grey region around them undergo complete readjustment, out of which emerges a characteristic spindle upon which are arranged the chromosomes in late prophase'. After division of the chromosomes the nuclear membrane constricts to form two daughter nuclei and the chromosomes apparently become more closely grouped to form the '*Councilmania*' nucleus, and chromatin returns to the peripheral layer. He does not, however, describe the late stages in great detail.

Wenrich stained his material with iron alum haematoxylin, haemalum, Delafield's haematoxylin, and by the Feulgen technique. He described the formation of two groups of chromosomes. The first group, which was composed chiefly of the karyosome material, elongated, and migrated towards the poles. As these two groups separated the peripheral and sub-peripheral chromatin migrated towards the centre and formed a second group of chromosomes. These also elongated and migrated to the opposite poles. The nuclear membrane eventually constricted in the centre, giving rise to two daughter nuclei.

According to Wenrich, Feulgen's 'nucleal reaction' stained the karyosome and the area surrounding it, but did not stain the peripheral layer of chromatin. By using this reaction, he claimed that the first group of chromosomes, which were formed from the karyosome, were stained whilst the second group were not stained, but were coloured by the counter stain. He also found that Delafield's haematoxylin stained the peripheral chromatin but not the karyosome, and that this technique selectively stained the second group of chromosomes. By this means the first and second groups of dividing chromatin granules could be identified during division.

As Feulgen's reaction appeared to be of great value these experiments were repeated. Wenrich does not give details of the procedure, but in a later paper on protozoological technique (1941*b*) he gives a reference to the method used (McClung, 1937), and it was assumed that this was used in the previous paper.

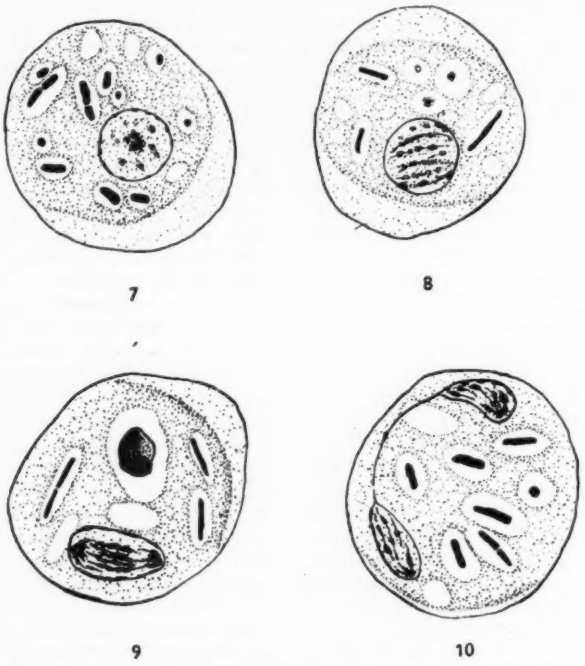
Using this and other methods, I have found that both the peripheral chromatin and the karyosome of the 'resting' nuclei of trophic amoebae remained uncoloured by Feulgen's stain. However, certain structures in dividing nuclei, and the karyosomes of recently divided binucleate individuals, were faintly stained with this technique. In view of this discrepancy details of these experiments are added.

Smears of caecal contents were fixed in Schaudinn's fixative and in sublimate-alcohol, and stored in 70% alcohol before use for not more than 7 days. Sodium sulphite, and potassium and sodium metabisulphite were used as reducing agents on different occasions. All solutions were prepared on the day that they were used except the leuco-dye solution, which in all but four experiments was 3 or 4 days old; in four

experiments it was 14 days old. The hydrolysis time was varied from 4 to 48 min. and the staining time from 2 to 17 hr.

The same reagents used with other material gave good results, thus the nuclei of trichomonads were well stained, whereas the nuclei of amoebae on the same smear were not stained. Increasing the acidity of the leuco-dye solution, and the hydrolysis time to 14–16 min. as recommended by Levitanskaïa (1938) for *E. coli*, was without effect upon *E. muris*.

In the course of mitosis, the chromatin granules observed during the prophase and metaphase, that is granules stained by the haematoxylin stains, appeared to originate from the karyosome, the peripheral layer, and from the area between these two structures. The karyosome divided to form a number of granules (Fig. 7) which varied from four to six in number (Wenrich describes four to eight), and these together with other granules formed four to six parallel threads of chromatin granules (Fig. 8).



Figs. 7–10. Nuclear division of *Entamoeba muris*. × 2000 (2 mm. = 1 μ).

Fig. 7. Karyosome of nucleus breaking up into granules, chromatin migrating from peripheral layer. S, H.
Fig. 8. Chromatin arranged in parallel threads. S, P.
Fig. 9. Nucleus elongating, polar caps distinct from other dividing granules. S, P.
Fig. 10. Daughter nuclei separated, but still connected by a chromatin thread. Karyosome in lower nucleus clearly separate and distinct from other chromatin. S, P.

In the present paper, nuclear division of *E. muris* was studied in material obtained from infected rats and mice, stained with Cooper's phosphotungstic haematoxylin, iron haematoxylin and by Feulgen's method. Dividing nuclei were extremely scarce, in fact not more than about ten individual amoebae with dividing nuclei were seen during the examination of a smear with the oil immersion objective, even when one to two amoebae were present in each field. Dividing nuclei were always more lightly stained than the 'resting' nuclei.

All these granules remained unstained by Feulgen's reaction.

The following stages observed showed that when the nucleus elongated, the nuclear membrane remained intact, and the dividing chromatin granules were arranged in three groups; a central group of granules arranged in a number of linear threads, and two polar groups of granules (Fig. 9). All the chromatin granules were stained with equal intensity by the haematoxylin stains, but the examination of Feulgen-stained smears showed that only the polar

granules were stained. This coloration was most intense in preparations which were hydrolysed for 6–8 min. and stained for 4 hr., but even so the coloration was very faint and of no value as a cytological stain.

The peripheral layer of chromatin had lost its beaded structure during the prophase and was present through the anaphase as a narrow very slightly beaded layer, until the reorganization of nuclear structure, when the more pronounced beaded structure reappeared. It remained entirely unstained by Feulgen's technique in both 'resting' and dividing nuclei.

The nucleus continued to elongate, and separated to form two daughter nuclei, these remained connected by a fine thread, which eventually disintegrated (Fig. 10). As the daughter nuclei separated, the Feulgen positive chromatin granules condensed and formed the karyosome, no other chromatin was observed to be incorporated into its structure. As these granules condensed the colour became more distinct, and the karyosome continued to exhibit a faint pinkish reaction for a short time after the nuclei had separated. It lost this property however, before the cytoplasm of the amoeba divided. The Feulgen negative granules appeared gradually to disintegrate and returned to the peripheral layer, which regained the typical beaded appearance.

The method of nuclear division described above, appears to be more complicated than that observed by Kessel. Structures observed by him, such as 'the dark grey region...around the granules of the dispersed karyosome', have been observed in iron haematoxylin preparations which proved to be over-differentiated, and it is thought that Kessel was misled by this artefact. Inspection of his figures, which show the absence of any granules in the nucleus other than the four or six 'chromosomes', also lends support to this view. Observations on cystic nuclear division are more difficult owing to variations in staining by individual cysts and to the presence of the glycogen vacuole which invariably distorts the nuclei.

The interpretation of the later stages of division observed agree with those of Wenrich in that the material destined to form the karyosome of the daughter nuclei appears to be separate from the other granules and that it alone is coloured by Feulgen's reagent. The earlier phases, before the nucleus has begun to elongate, are very puzzling, and the origin of the Feulgen-positive and Feulgen-negative chromatin was not discovered (it is doubtful if such faint coloration may be properly called positive). Stages such as that figured in this paper were observed (Fig. 12), and here it seems that chromatin from both the karyosome and peripheral layer has contributed to the mitotic figure almost simultaneously. No stages were seen in which two distinct mitoses were present as figured by Wenrich.

The transient response of the nucleus of *E. muris* to staining with Feulgen's reagent described above is also shown by *Endamoeba blattae* (Meglitsch, 1939) and has also been observed in *Trichomonas vaginalis* (Hawes, 1947). Meglitsch interpreted this phenomenon as an increase of free nucleic acids. Although the deepening of the colour in *Entamoeba muris* may be explained in a similar manner, it is also conceivable that it may be due to a condensation of weakly stained, diffuse elements.

(5) *Description of the cysts*

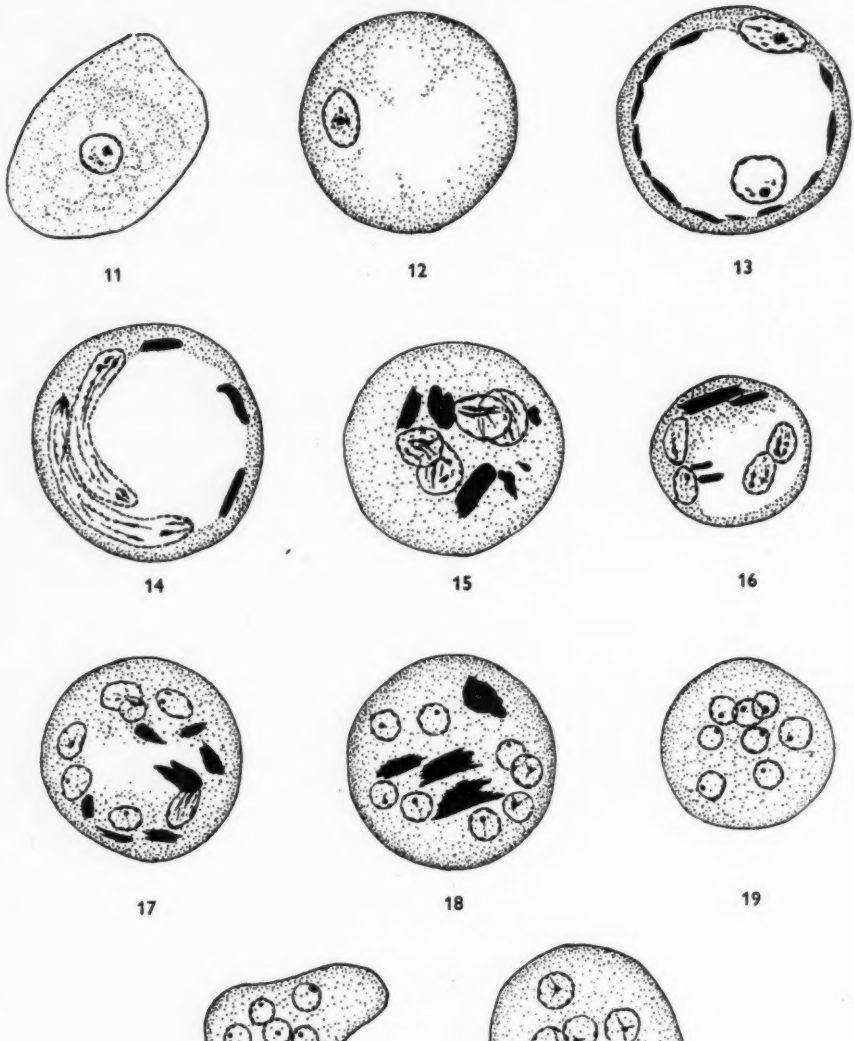
Precystic amoebae. These forms (Fig. 11) can be distinguished from the trophic amoebae by the nuclear structure which indicates a recent division, together with the absence of any inclusions in the cytoplasm. The cytoplasm consequently has a homogeneous structure, the ectoplasm not being so clearly demarcated from the endoplasm as in the trophic stages. The nucleus is smaller and the structure of the karyosome, together with the presence of chromatin granules between it and the peripheral chromatin layer, indicate a late telophase stage of division. These forms were never abundant in the material examined.

Encystment. The amoebae encyst and complete their development in the caecum of the host and pass out with the faeces. Normally only 8-nucleate cysts together with a few immature cysts are found in the faeces. The most easily observed stages of encystation are the binucleate and 8-nucleate cysts, while the intermediate forms are more difficult to discover. No cytological evidence was obtained in support of Wenrich's suggestion (1940) that the amoebae encysted at the binucleate stage.

Shape. The cysts are usually approximately spherical in shape, though they are rarely exactly spherical, and may be oval or slightly distorted. Irregularly shaped cysts are infrequent but may occur in larger numbers in individual hosts (Fig. 20).

Glycogen. The glycogen vacuole usually appears as soon as the uninucleate cyst is formed (Fig. 12). At this stage, the outline of the vacuole is often indefinite and cytoplasmic processes protrude into it, indicating that the vacuole was probably formed by the coalescence of a number of smaller vacuoles. It reaches maximum size at the binucleate stage (Figs. 13, 14), when the cytoplasm is limited to a narrow zone against the cyst wall, and the nuclei are distorted to an oval shape. As the development of the cysts proceeds, the size of the vacuole diminishes and it usually disappears when the 8-nucleate stage is reached.

This vacuole stains brown with Lugol's iodine and



Figs. 11–21. Cystic stages of *Entamoeba muris*. × 2000 (2mm. = 1 μ).

pink with Best's carmine stain, indicating the presence of glycogen.

Chromatoid bodies. These structures appear in the binucleate cyst at the edge of the glycogen vacuole and are generally in the form of bars with pointed ends. Chromatoid bodies are often absent from the mature cyst but when present they are larger than those in binucleate cysts and may be of any shape, but are usually bar-shaped with irregular, splintered or less frequently, rounded ends. Rarely they are rounded in shape (Figs. 13–18, 20 and 21).

Cystic nuclei. The formation of 8-nuclei in the mature cyst takes place by three almost simultaneous divisions (Figs. 12–18). Supernucleate cysts containing up to 16 nuclei are occasionally observed; these cysts are usually larger than the normal size. Multiplication of the nuclei results in a diminution in size, the diameter of the nuclei of the mature cyst varying between 2 and 3 μ.

The nuclei are faintly visible in the living cyst lying in the cytoplasm and are more clearly observed by staining with iodine solution. The structure of the nuclei of the mature cyst is identical to that of the trophic amoebae, that is, the peripheral chromatin is arranged in fine granules equal in size, more or less touching one another, and the karyosome is represented by a small granule varying in position from extreme eccentricity to the centre of the nucleus. A few smaller granules may be observed lying between the karyosome and the peripheral chromatin.

Size. The size of the cysts was studied in nine natural infections of *E. muris*, and in experimental infections in rats and mice. Two hundred 8-nucleate cysts from each infection were stained with Lugol's iodine solution, and measured by means of an ocular micrometer, one division of which represented 1·4 μ. The preparations were sealed with Czokor's wax to prevent evaporation, and the results were plotted graphically, with cyst diameters as abscissae and the frequencies as ordinates

The mature cysts vary in size from 9 to 23 μ in diameter, but supernucleate cysts were generally larger; thus one cyst containing sixteen nuclei measures 29 μ. Fulton & Joyner (1948) recorded cysts measuring up to 24 μ. When the frequencies were plotted graphically, unimodal histograms were obtained; the modal point was situated at 13·9 or 16·7 μ, which indicated the presence of two races.

Fig. 22 shows two such graphs, one from infection in a mouse and the other from a rat. These histograms differ significantly from one another ($z = 2{\cdot}1$, $P > 0{\cdot}01$). Table 1 gives a statistical analysis of data presented graphically in Fig. 22.

Table 1. *Statistical analysis of data shown in Fig.* 22

	No. of cysts measured	Range (μ)	Mean $\pm$ standard error (μ)
Rat	200	12·5–22·2	16·89 $\pm$ 0·10
Mouse	200	9·3–19·5	14·20 $\pm$ 0·10

Cysts from a laboratory mouse were passaged through clean rats (methods of passage are described below) and then through rats again for a second and third passage. After each passage 100 mature cysts were measured as described above. The results are shown as polygons in Fig. 23.

These results show that the cysts did not change significantly in size during three passages. Unfortunately, the cysts from the original mouse were too scanty in number to obtain a sufficient number of measurements. The polygon shows that it was the same size as the small race (Fig. 22*A*), therefore it is probable that the size of the cysts had not altered during the transference to a different host.

Cysts of the larger race from rats were transferred to mice, and 100 cysts produced by the resulting amoebic infection were measured. These results (Fig. 23*B*) demonstrated that the size of the cysts of the larger race remained constant when transferred to the opposite host for one passage.

Legends to Figs. 11–21

Fig. 11. Precystic amoeba from a rat, with absence of differentiation of ectoplasm and endoplasm, nucleus in late division phase. S, P.

Fig. 12. Uninucleate cyst from a rat, with the glycogen vacuole in the process of formation. S, P.

Fig. 13. Binucleate cyst from a rat, with fully formed glycogen vacuole surrounded by chromatoid bodies. S, P.

Fig. 14. Binucleate cyst from a rat, with dividing nuclei. S, P.

Fig. 15. Large 4-nucleate cyst from a rat, with dividing nuclei, few chromatoid bodies, glycogen vacuole absent. S, H.

Fig. 16. Small 4-nucleate cyst from a mouse, with bar-shaped chromatoid bodies and a glycogen vacuole present, S, H.

Fig. 17. Cyst with seven nuclei from a rat, division of one nucleus delayed, small glycogen vacuole still present. S, H.

Fig. 18. Typical mature, 8-nucleate cyst from a rat, showing chromatoid bodies, large race. S, H.

Fig. 19. Typical mature 8-nucleate cyst from a mouse, small race, without chromatoid bodies. S, P.

Fig. 20. Irregularly shaped, 8-nucleate cyst from a mouse. S, H.

Fig. 21. Large oval 8-nucleate cyst from a rat, without chromatoids. S, P.

Both races of *E. muris* were discovered in naturally occurring infections of laboratory mice, but only the larger race was observed in natural infections of rats. In view of the ease of cross-infection, and the morphological identity of these races, however, it was concluded that both races could parasitize rats and mice with equal ease. Kessel's observations on the size of the cysts confirm this point.

The occurrence of distinct races in murine amoebae has been previously recorded by Kessel (1924), who found three races, all of which occurred both in rats and mice, but did not publish any details of the investigation. He found the size of the smallest race to be 14·5 μ (*Councilmania muris*) and 14·0 μ (*C. decumani*), whilst the largest race measured 17·7 μ (*C. muris*) and 17·0 μ (*C. decumani*): cysts of *Endamoeba ratti* measured 14·4 μ and were found only in rats.

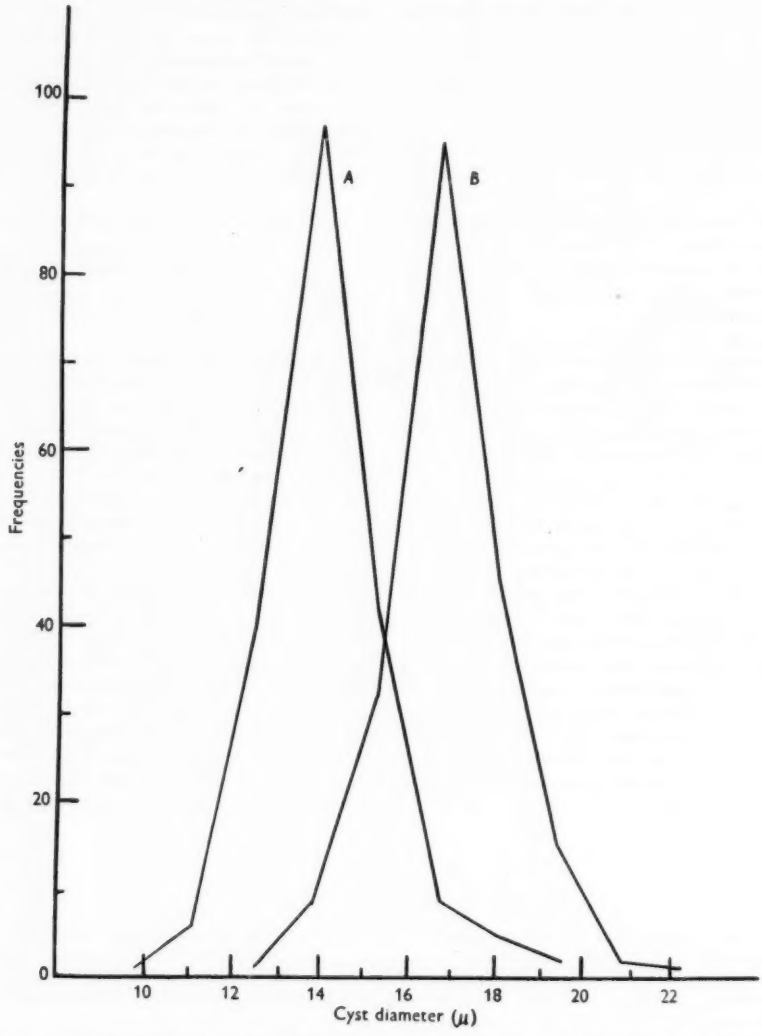


Fig. 22. Histogram and polygon of size of cysts of *E. muris* showing the presence of two races. A, *E. muris* cysts from a mouse. B, *E. muris* cysts from a rat.

The data recorded above agree with Kessel's figures and confirm the existence of at least two races, distinguishable by the size of the ripe cysts. No representative of his medium-sized race which measured 15·7 μ (*C. muris*) and 15·5 μ (*C. decumani*) was observed by me.

parations. In some cases, however, no allowance was made for artefacts which may have arisen during the preparation of permanent slides.

Wenyon (1907) gave a good description of the amoebae found in mice. However, this included an account of autogamy, in which he confused the

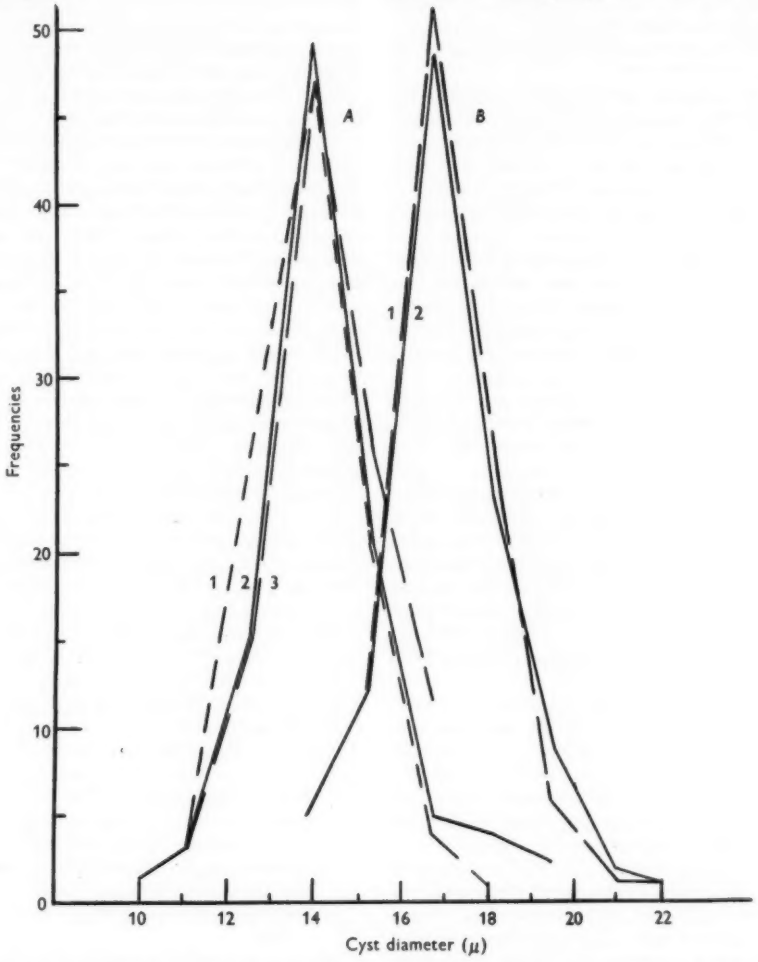


Fig. 23. Polygons showing the size of the cysts of *E. muris* after *in vivo* passage. A, the size of a mouse strain of *E. muris* after three passages (1, 2 and 3) in rats, showing no change in size of the diameter. B, comparison of the size of cysts of a rat strain of *E. muris* in the original host (1) and after passage in the mouse (2), demonstrating no change in size.

(6) *Discussion of the morphological characters of* Entamoeba muris

The morphology of the amoebae of rats and mice, previously described by various authors, was based principally on the study of iron haematoxylin pre-

chromatoid bodies with nuclear chromatin and figured them being thrown out of the cyst. Through the courtesy of the late Dr C. M. Wenyon, his original preparations were examined, and they were found to be identical to the amoebae described in the present paper.

The presence or absence of chromatin granules between the karyosome and peripheral chromatin was thought by Rudovsky (1921) to be of diagnostic importance. In the present investigation it was found that this feature was not constant. Differences in the thickness of the peripheral chromatin between amoebae occurring in rats and those found in mice were not observed; these differences may possibly have been due to artefacts produced by staining, as noted above.

In order to consider the classification given by Kessel (1924), it is necessary to discuss the origin and status of the genus *Councilmania*. The genus *Councilmania* was established by Kofoid & Swezy (1921) for a pathogenic amoeba, *C. lafleuri*, found in man. It was distinguished on morphological grounds from *Entamoeba coli* by the presence of ectoplasmic pseudopodia, *E. coli*, being supposed to have endoplasmic pseudopodia only, and from both *E. coli* and *E. histolytica* by the structure of the karyosome, which was composed of a number of more or less discrete granules occupying a central position, and by a process of 'budding' from the 8-nucleate cyst. The taxonomic value of this genus was severely criticized by Wenyon (1925), Wight & Prince (1927), and others, on the grounds that these characters were either artefacts produced by fixation, or that the infection from which the genus was described was a mixed one of *E. coli* and *E. histolytica*. Later Stabler (1932), in an extended study of the variation in morphology of *E. coli* in a single case, observed that the amoebae and cysts presented the characteristics of *E. coli* and *Councilmania lafleuri*. It has since been shown by Pavlova (1938) that the structure of the pseudopodia is not constant enough to be of generic or specific status.

Kessel (1924) observed that some of the rodent amoebae protruded ectoplasmic pseudopodia and as these, together with other features, were characteristics of the genus *Councilmania*, he accordingly classified them as species of this genus, while the amoebae which moved sluggishly were placed in the species *Endamoeba ratti* (= *Entamoeba ratti*).

As already stated above (p. 345), *Entamoeba muris* is similarly sensitive to environmental changes, but when observed under favourable conditions almost all amoebae show clear ectoplasmic pseudopodia. Also in fixed and stained smears, the different nuclear characters, which were described by Kessel to be associated with the two types of pseudopodia, were not observed. Indeed the dispersed type of karyosome has not been found in normal amoebae in the present investigation except in nuclei undergoing division, but such stages were remarkably rare (see p. 348). Cysts of *E. muris* with buds were rarely observed in preparations made with saline or iodine but were encountered more frequently in material fixed with Schaudinn's solution. There can be no doubt that budding observed under these conditions is an artefact, rather than a natural phenomenon.

The close agreement in size of the trophic amoebae and of the cysts of *Councilmania muris* and *C. decumani* belonging to three races, is suggestive of the identity of these two species—as Kessel later (1930) admitted—and also of *Entamoeba ratti*. *Councilmania muris* and *C. decumani* are accordingly rejected as invalid species and become synonyms of *Entamoeba muris*, while *E. ratti* is indistinguishable from *E. muris*, as described above, and is a synonym of *E. muris* on grounds of priority.

An amoeba having the structure of that described by Chiang (1925*a*,*b*) was not observed. He claimed that it was biologically distinct from *Endolimax nana* as he was unable to infect rats with *E. nana* of human origin. However, Kessel (1923) and Smith (1928) were able to transfer *E. nana* to rats, although the number of rats experimentally infected was small (two out of twenty-five infected, and four out of sixty-three infected respectively). Chiang in the same papers reported upon the discovery of *Entamoeba histolytica* in laboratory rats and it seems probable that some of his animals must have had access to material containing cysts of *E. histolytica* and therefore possibly those of *Endolimax nana*. Further evidence is required before *E. ratti* can be accepted as a distinct species.

It is thus seen that there is no morphological evidence suggesting the presence of more than one species of non-pathogenic amoebae parasitic in rats and mice. In all stages of the life history of *Entamoeba muris* which have been studied, it is not possible to distinguish this species from *E. coli* (see also p. 363). For this reason, the division of the amoebae of rodents into two species, *E. muris* and *E. coli* var. *ratti*, by Epstein & Matevosjan (1929) and Epstein (1941), is unfounded.

The following species have therefore been shown to be synonyms of *E. muris* (Grassi, 1879):

Amoeba muris Grassi, 1879.
Entamoeba muris decumani Rudovsky, 1921.
Councilmania muris Kessel, 1924.
Councilmania decumani Kessel, 1924.
Endamoeba ratti Kessel, 1924.
Entamoeba coli var. *ratti* Epstein & Matevosjan, 1929.

The relationship of *E. coli* and *E. muris* is further discussed (p. 362), after a description of the results of experimental infections of rats and mice with these species of *Entamoeba*.

III. EXPERIMENTAL INFECTIONS WITH *ENTAMOEBA MURIS*

Experimental infections of rodents with *E. muris* have been attempted by Wenyon (1907) and Kessel (1924). Wenyon described the successful infection of

one mouse, previously found to be negative by 'repeated examinations', with cysts from another animal of the same species. In a more extensive research, Kessel (1924) found that cysts obtained from an amoebic infection in a rat infected four out of twelve mice examined 2 months after inoculation, and conversely cysts from mice infected six out of seven rats examined 3–4 weeks after inoculation.

Kessel's experiments are, however, open to criticism. First, the long period elapsing between the feeding of the cysts and autopsy is sufficient to allow a single infected animal (whether an undetected natural infection or an experimental infection) to pass cysts and infect others housed in the same cage. Secondly, the positive results may have been due to contamination from outside sources as no precautions to prevent this occurrence are described. In either case a fallacious result would be obtained. In view of these criticisms, these experiments were repeated.

(1) *Material and methods*

(*a*) *Experimental conditions.* In this work, experiments were at first conducted without any special precautions to prevent accidental contamination by amoebic cysts from other animals, that is, employing methods similar to those apparently used by Kessel (1923, 1924), and others. It was soon realized that rigid experimental conditions were necessary to prevent accidental infection and all experiments were eventually conducted with the following precautions.

The animals were caged singly, and each cage was placed separately upon a tray, each animal being separated from others by at least 12 in. The bedding (sawdust and wood-wool) was sterilized by dry heat (180° C.) for 1 hr. and the animal cages steamed for 30 min. The sawdust was changed on alternate days.

The animals were fed upon a dry mash, which was thoroughly dried before feeding, by heating it for 48 hr. at 50° C.; the dried diet used for feeding infected animals was separate from that used for uninfected animals. Water was available *ad lib.* from licking bottles, and these were filled with water from the hot tap.

The experimental animals were kept in a small room completely isolated from all other laboratory animals. It was endeavoured to conclude each experiment before the commencement of another, but in a few cases 1 or 2 days overlap was unavoidable. No cockroaches or other possible carriers of cysts were seen in this room.

Contamination of animals by 'foreign' faecal pellets was thus reduced to a minimum, although it was possible for them to ingest fragments of their own faeces; however there was no evidence that they practised coprophagy.

(*b*) *Methods of examination of material for protozoa.* For the detection of *E. muris* in caecal material, fresh smears were prepared with normal saline or Ringer's solution, and if necessary a second smear was made using Lugol's iodine solution, while smears of faeces were always made in iodine solution. The smears were covered with a $\frac{3}{4}$ in. cover-slip and examined with the 4 mm. objective and a 10× or 6× ocular. A microscopical examination recorded as negative means that the entire smear was examined with the aid of a mechanical stage without finding any amoebae or cysts. The examination of a single smear occupied about 15 min.

An arbitrary scale was devised for recording the approximate number present (see Table 2).

Table 2. *Arbitrary scale used for recording the number of amoebae or cysts of* Entamoeba muris *present in a smear examined with the* 4 *mm. objective*

No. observed	Symbol
1–6 in the entire smear	±
7–20 in the entire smear	+
1–5 per 20 fields	++
6–20 per 20 fields	+++
More than 1 per field	++++

Faeces, obtained without the use of a purgative, were collected in sterile test-tubes.

(*c*) *Treatment of cysts for infection of animals.* The cysts were concentrated by a method devised by Dobell & Laidlaw (1926). The material containing cysts was emulsified in about 100 ml. of 1/2-strength Ringer's solution or 1/2-strength normal saline solution, and the suspension was allowed to settle for 5 min. in a 100 ml. measuring cylinder. The supernatant liquid containing the cysts was decanted into another cylinder and was allowed to settle overnight, the cylinder being placed in the refrigerator (4° C.). The whole operation was then repeated the following day using the sediment at the bottom of the measuring cylinder. This procedure could be repeated many times, though in practice the cysts were given three washings, as some of the deposit was unavoidably lost in each washing.

The cysts were stored in the refrigerator (4° C.) until they were required. The number of cysts per volume in a suspension was determined with a haemocytometer.

By this method, the cysts were concentrated in the deposit without damaging them in any way. The deposit, which consisted of cysts, small faecal particles and a variable number of bacteria, was easier to handle for inoculating animals than the original material, and fungi grew less readily when it was stored in the refrigerator for long periods.

(*d*) *The strains of animals and the methods used to detect spontaneous amoebic infections.* The rats used in the present work were albinos, Wistar strain

(*Rattus norvegicus*). They were all obtained from the same source and all were isolated under the above conditions at 18–21 days old. It was not found necessary to feed the young rats with milk as recommended by Ratcliffe (1929), although it was sometimes advisable to assist them at first to drink from the licking bottle.

The rats were first examined by one microscopical examination of material taken directly from the caecum, to detect any spontaneous infections of *Entamoeba muris* (the technique of this procedure is similar to that described by Goodwin *et al.* 1948). In later experiments, in particular those dealing with experimental infections of *E. muris*, faecal examinations were combined with caecal examinations, the exact number of which is stated in each experiment.

The longest period during which it was attempted to keep rats free from amoebic infection was 17 weeks. During this period sixty-seven faecal examinations were made upon three rats with negative results. The incidence of *E. muris* infections in newly weaned rats is discussed later (p. 361).

The mice used for *E. muris* cross-infection experiments reported below were from a single litter of Swiss mice (*Mus musculus*), which were separated from the doe at the age of 18 days. The faeces of the doe were examined on 4 consecutive days, without any amoebic cysts being found. The faeces of the young mice were examined for 6 consecutive days, and the caecal contents on the 7th day, but no amoebae or cysts were observed.

Other work has shown that natural infections of mice with *Entamoeba muris* sometimes escape detection, when only two microscopical examinations of caecal material are performed. In view of this observation, it seems possible that the method used by Kessel (1923, 1924) and by Regendanz (1929) would overlook some infections of *E. muris*. However, the mice and rats used in the present investigation, which were weaned at the earliest possible moment and kept isolated under the conditions described above, were not found to be naturally infected with *E. muris*, even when the faeces as well as caecal contents were examined for a period of weeks.

The animals were not always entirely free from other protozoa, the following of which were occasionally observed: *Trichomonas parva*, *T. muris*, *Chilomastix bettencourti*, *Giardia muris* and *Hexamita muris*. The presence or absence of these species is not given in the following description, but full details are recorded in my notes.

(2) *Infection of mice with a rat strain of* Entamoeba muris

The faeces of a laboratory rat containing mature 8-nucleate cysts were collected and washed three times. About ten to fifteen cysts, which were 10 days old, were administered orally to two mice which were 59 days old, by a metal catheter. The faeces of these mice had been examined on twenty-two occasions, and material from the caecum once, without finding any cysts or amoebae during a period of 34 days. The probability is high that these animals were not infected with *E. muris* prior to the experimental infection.

The results of the examination of the faeces of these animals for cysts after inoculation with *E. muris* is shown in Table 3.

Table 3. *Exp.* 64B. *Results of attempts to infect mice with a strain of* Entamoeba muris *from a rat*

	No. of cysts observed in the faeces. Days after inoculation													
Mouse	1–5	6	7	8	9	11	12	13	14	21	29	30	33	35	36
R	—	±	±	—	—	—	+	—	—	—	—	+	±	—	.
2L	—	—	±	±	—	—	—	.	.	.	.	.	.	.	.

Cysts were first detected in the faeces of mouse R and 2L on the 6th and 7th days respectively after inoculation, and mouse R continued to pass them in the faeces, although never in large numbers. Mouse R was killed on the 36th day after infection and amoebae (+ +) were observed in the caecum, whilst mouse 2L was killed on the 14th day and only a few (±) amoebae were seen. Both mice were in good condition and the intestines were macroscopically normal. Stained smears of the caecal contents showed normal amoebae.

(3) *Infection of rats with a mouse strain of* Entamoeba muris

Faeces containing cysts were collected from a laboratory mouse naturally infected with *E. muris*, were washed once, and after having been kept in a refrigerator for 3 days, about 30 cysts were inoculated orally by metal catheter to two rats. During the previous 42 days the faeces of the rats (which were 9 weeks old at the time of inoculation) had been examined twenty-six times and material from their caeca twice, without observing cysts or amoebae of *E. muris*.

Table 4. *Exp.* 60. *Results of attempts to infect rats with a strain of* Entamoeba muris *from a mouse*

	No. of cysts observed in the faeces. Days after inoculation						
Rat	1–4	5	6	8	9	10	11
O	—	—	—	—	—	±	±
R	—	±	—	±	—	±	—

After inoculation, cysts of *E. muris* were first observed in the faeces of rat R on the 5th day and in

the faeces of rat O on the 10th day (see Table 4). Cysts continued to be passed until the 14th day when the animals were killed. Both rats were perfectly healthy and the amoebae found in the caecum were morphologically identical to those described above.

(4) *Infection of rats and mice with a hamster strain of* 'Entamoeba muris'

The caecal contents of a hamster (*Cricetus auratus*) containing numerous cysts were collected, the cysts washed three times, and stored in the refrigerator for 31 days. The cysts were then administered orally to three rats, each receiving approximately fifty cysts. The rats were 32 days old at the time of inoculation, and their faeces had been examined eight times and the caecal contents twice during the previous 11 days without observing *E. muris*. The results of examinations of these animals after inoculation are shown in Table 5.

Table 5. *Exp.* 62. *Infection of rats with a hamster strain of* 'Entamoeba muris'

	No. of cysts observed in the faeces. Days after inoculation													
Rat	1–7	8	9	11	12	13	14	15	18	23	25	26	27	28
L	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2R	—	+	±	—	—	+	—	±	—	—	±	+	—	+
2L	—	+	—	—	±	—	—	±	—	—	±	—	—	.

Cysts were first detected in the faeces of rats 2R and 2L on the 8th day and continued to be passed subsequently. Caecal material was examined on the 19th day and amoebae observed in rats 2R and 2L. Rat 2L was killed on the 29th day and amoebae (±) were found in the caecum. Rat 2R remained infected for 12 weeks, after which no more cysts were discovered. No amoebae or cysts were detected at any time in the third rat (L) and it must be presumed that it was not infected. Two normal rats, which served as controls, remained negative throughout the experiments.

Faeces containing cysts from rat 2R in the above experiment (Exp. 62) were collected and washed three times, and two mice inoculated orally with cysts, after the cysts had been kept 58 days in the refrigerator. These mice had been examined for *E. muris* eight times (seven faecal and one caecal examination) with negative results in the previous 9 days.

Table 6. *Exp.* 64A. *Infection of mice with a hamster strain of* 'Entamoeba muris' *from rat* 2R, *Exp.* 62

	No. of cysts observed in the faeces. Days after inoculation												
Mouse	3	4	5	6	7	8	12	13	14	17	18	20	21	23
RL	—	+	—	—	±	—	±	±	—	—	±	—	.	.
2R	±	±	—	—	++	±	+	+	±	—	.	—	.	.

Cysts were first observed on the 3rd (mouse 2R) and the 4th (mouse RL) day after inoculation (see Table 6). Cysts continued to be passed, and a caecal examination on the 19th day revealed the presence of amoebae. Mouse RL was killed on the 21st day after inoculation and amoebae (++), which were observed in the caecum, were morphologically identical to those described above. Mouse 2R has continued to pass cysts, and up to the time of writing (23 August 1949) has remained infected for 40 weeks. Three control mice remained negative throughout the experiments.

The dimensions of the cysts from an animal in each experiment (Exp. 62, rat 2R; Exp. 64A, mouse 2R) were compared with those of the original hamster material. It was found that the dimensions were in good agreement within the limits of experimental error.

(5) *Observations on infections with* Entamoeba muris

The course and duration of experimental infections with *E. muris* were followed by faecal examination of five infected animals. Cysts were first observed in the faeces of rats and mice 3–10 days after inoculation. The presence of immature as well as 8-nucleate cysts indicated that they were not cysts of the inoculum which had passed through the intestine without hatching. The number of cysts observed at each examination varied from ± to +++ in various infections, and periods of up to 6 days, during which no cysts were detected, were encountered between positive examinations when daily observations were made. On most occasions only mature, 8-nucleate cysts were observed, but infrequently immature cysts with large glycogen vacuoles were seen; amoebae were found in the faeces only if the normal functioning of the large intestine had been impaired, producing diarrhoea. In two mice, an irregular periodicity was observed, the number of cysts passed in the faeces fluctuating on different days from zero to +++, while in other animals this phenomenon was not observed.

If a method had been available for cultivating *E. muris*, it is highly probable that it would have been possible to demonstrate the presence of *E. muris* in specimens for which negative results are recorded.

The infections were followed by faecal examinations three times a week, and it was found that the cysts disappeared from the faeces after a variable period of time (Table 7).

The faecal examinations of rat O, Exp. 51, and of mouse L, Exp. 50, remained positive for 8 and 17 weeks, but were unfortunately interrupted for 5 and 3 months respectively, and when examinations were resumed they were found to have lost their infections.

Table 7. *The duration of infection with* Entamoeba muris

Origin of infection	Host	Duration	No. of negative examinations after last positive
Natural	Mouse	At least 17 weeks	23
Exp. *E. muris* from rat	Rat	At least 8 weeks	26
	Rat	10 days	32
Exp. '*E. muris*' from hamster	Rat	12 weeks	31
	Mouse	At least 40 weeks	—

In four infections with *E. muris* (Table 7), it was found that cysts eventually disappeared from the faeces in the animals studied. No cysts were found subsequently, even when examined thrice weekly for periods longer than 2 months, nor were amoebae found in the caeca at autopsy. It is difficult, of course, to decide if in such infections the amoebae had truly died out; this can only be ascertained by prolonged observation, but in these particular cases I believe the period of examination was sufficient to justify this conclusion. One animal, mouse 2R (Exp. 64A), was infected with a hamster strain of '*E. muris*' on 9 November 1948 and is still passing numbers of cysts in its faeces (22 August 1949). There was no difference between infections with any of the strains of *E. muris* (rat, mouse, or hamster) used in the experiments. The fact that infections with *E. muris* tend to die out is also shown by the decrease of the incidence of infection among laboratory rats as the age of the animals increases (Kessel, 1924). Kessel also states that 'old rats and mice are less susceptible to infection than young stock', but he produces no evidence in support of this statement.

Cysts of *E. muris* were kept at 4° C. for as long as 84 days without losing their viability, whereas in one experiment when cysts were kept at 4° C. for 103 days, negative results were obtained in attempting to infect four rats with cysts of this age.

The distribution of *E. muris* in the alimentary canal was studied by removing material from four distinct parts of the alimentary canal of thirteen rats positive for *E. muris*; the blind apex of the caecum, the caecum opposite the entrance of the ileum, the colon, and the ileum above the ileo-caecal valve. These are termed positions A, B, C and D. Amoebae were observed on ten occasions in material from position A, thirteen from position B, seven from position C, and none from position D. In three animals there were fewer amoebae from position A than B. In heavy infections no difference was observed in the numbers of amoebae from the different positions. In the rectum, where the intestinal contents were solid and formed into pellets, only degenerate amoebae were observed. Cysts were observed in the caecum, colon and rectum. Similar results were observed with mice.

These observations show that *E. muris* inhabits the caecum of rats and mice and that the amoebae degenerate and die after leaving the colon. The reason for the apparent greater numbers of amoebae at the entrance of the ileum to the caecum is obscure, but it may be that some unknown food factors encourage more rapid multiplication or that the denser contents of the blind apex slow down the wandering of amoebae to this region.

The absence of amoebae above the caecum is of interest, since Harkema (1936) and Kofoid, McNeil & Bonestell (1933) claim to have found amoebae (classified as *E. muris*) in the small intestine of the rat. It is possible that post-mortem wandering of amoebae may explain these anomalous findings, although in the case of Harkema, who discussed the incidence of *E. muris* in wild rodents, it may also be a clerical error.

E. muris is non-pathogenic, for no lesions were observed in any infected animals, even when heavy infections were encountered.

IV. ATTEMPTS TO INFECT RATS AND MICE WITH *ENTAMOEBA COLI*

In attempting to evaluate the taxonomic relationship of *E. muris* and *E. coli*, it is essential to determine the degree of host restriction of these parasites for rats and mice. Kessel (1923) found that, on feeding *E. coli* cysts from man to ten rats, three of them developed an amoebic infection. Kessel in the paper describes other experiments such as the results of feeding '*Councilmania lafleuri*' cysts to rats, but unfortunately owing to the fallacy in his method of obtaining rats free from natural infections of *Entamoeba muris*, and to the uncertainty regarding the species of amoebae present in the faecal material in the first instance, in addition to the criticisms of his experimental methods (see p. 355) it is impossible to assess the value of his experiments.

Later Regendanz (1929), using methods similar to those of Kessel, observed that *E. coli* could parasitize rats if amoebae or cysts were given by the rectal route, but not if given orally. Chiang (1925*a*, *b*), on the other hand, fed rats with faecal material containing *E. coli* cysts and did not observe any amoebic infection at autopsy.

Most of the present work was performed using rats as experimental hosts, since from an ecological viewpoint, these animals are more likely to come into contact with *E. coli* than mice.

(1) *Material and methods*

In general, the methods used were identical to those described above (p. 355). Exps. 8–36 were not

conducted with the strict precautions described, but have been included in the following description as the results are entirely negative.

The rats were identical to those described above, but were examined by at least one caecal examination.

The mice used were pure-bred albino, Stoney strain (*Mus musculus*), aged about 3–4 weeks. These mice were not all free from natural infections of *Entamoeba muris*, the incidence varying with different batches of animals. The mice were examined twice by the removal of material from the caecum, with approximately 7 days between each examination. Animals with two negative microscopical examinations of caecal material were used for experimental infections.

Although this method for obtaining animals free from *E. muris* is not completely reliable when used alone, the negative results obtained in trying to infect mice with *E. coli* were, with one exception (see below), negative.

Human faeces containing mature cysts of *E. coli* were treated as for *E. muris* cysts. The faeces were obtained from a number of sources, but most were obtained from Mr A. V. H. Allen of the Hospital for Tropical Diseases.

Three cultivated strains of *E. coli* were used. Strain GE was isolated and maintained *in vitro* by me, and was cultivated from material in which only amoebae were seen. The presence of a pure strain of *E. coli* was confirmed on morphological grounds. This strain grew with great regularity and was thoroughly established *in vitro*. Culture amoebae and cysts of the two other strains of *E. coli*, MC and MD, were received from Dr Dobell. Strain MC was isolated from a marmoset (*Hapale jacchus*), and was successfully transferred to macaques (*Macacus sinicus* and *M. rhesus*) and to man (Dobell, 1936). Strain MD is the derivative of MC in man and was recovered from man *in vitro* on 26 December 1948.

Cultural methods of examination for *E. coli* were made in HSre medium (Dobell & Laidlaw, 1926) with rice starch, and the results were determined by examination after 24 and 48 hr. incubation at 37° C.

(2) *Attempts to infect rats with* Entamoeba coli *from man*

Mature cysts of *E. coli* were administered orally to young rats and these were autopsied after a variable number of days, and the caecal contents were examined microscopically.

With one exception, the results were completely negative. As stated previously, Exps. 8–36 were not conducted under the strict conditions described above, and it is therefore possible that this positive result (Exp. 22, amoebae were found in one rat out of four inoculated) may have been due to *E. muris* rather than *E. coli*.

A summary of these experiments is shown in Table 8.

Table 8. *Summary of attempts to infect rats with* Entamoeba coli *cysts from man*

Exp.	Strain	No. of rats inoculated
8	Watson	10
17	Smith	9
22	Allen	4
31	Francis	4
32	M–J	5
36	Tony	5
37	Whales	1
38A	Aldridge	4
38B	Chaplin	4
42	Nana	2
53	Rowley	2

The experimental details were modified in various ways. The rats were killed after 6–20 days had elapsed after inoculation, and the number of cysts given to each rat was varied from 10 to 300. Doses of *E. coli* cysts were given on consecutive days (Exps. 37, 38A and 38B), and in Exp. 42 the cysts were inoculated directly into the caecum, after laparotomy. In the latter experiment, four litter-mate rats were divided in two equal groups and after the rats inoculated with *E. coli* cysts had been killed and found to be negative, the two litter-mates were successfully infected with cysts of *E. muris*. Increasing the carbohydrate content of the food also had no effect in establishing an infection with *E. coli* (Exp. 8).

An attempt was made to infect seven rats with *E. coli* amoebae by injecting intra-caecally a faecal suspension containing a large number of *E. coli* amoebae. All seven rats were negative at autopsy 7 days after inoculation (microscopical and cultural examinations). The material was 90 min. old when inoculated and gave positive *in vitro* cultures after 24 hr. incubation at 37° C.

(3) *Attempts to infect rats and mice with cultured strains of* E. coli

Culture amoebae of strains GE and MC were injected intracaecally after exposure of the caecum by laparotomy into rats and mice. Half of the deposit containing starch and amoebae of each tube were injected into each animal (about 0·3 ml. in volume), immediately after removal of the amoebae from the culture tube. No determination was made of the number of amoebae but many hundred amoebae must have been present. Sub-cultures of the remainder of the material after the inoculation of animals, showed good growth after 24 hr. incubation

at 37° C., thus demonstrating the viability of the inoculum. MC strain was inoculated into four rats and three mice, and GE strain into four rats and five mice. The animals were autopsied 5–24 days after inoculation, and the caecal contents were examined microscopically and culturally.

The autopsies demonstrated that one rat was infected with GE strain, whereas all other animals were negative. In this case (Exp. 47) the amoebae observed in the rat at first grew well *in vitro* but the contained large numbers of cysts, which when incubated in suitable media at 37° C. hatched, and developed normally. The cysts were stored in the refrigerator for 7 (Exp. 54), 10 (Exps. 56 and 66), 49 (Exp. 59) and 73 (Exp. 63) days. The faeces of these animals were examined microscopically and culturally for various periods after inoculation (see Table 9), and the contents of the large intestine were similarly examined at autopsy. All these experiments were completely negative, a summary of the results is given in Table 9.

Table 9. *Summary of attempts to infect rats and mice with* Entamoeba coli *culture cysts*

Exp.	Lab. host and strain	No. of animals inoculated	No. of negative examinations after inoculation
54	MC, mice	2	8
56	MC, rats	2	4
59	MC, rats	3	8
63	MC, rats	2	21
66	MD, rats	3	29

V. INCIDENCE AND GEOGRAPHICAL DISTRIBUTION OF *ENTAMOEBA MURIS*

(1) *Incidence*

The incidence of *E. muris* in wild rats reported in the literature (Table 10) where 100 or more animals have been autopsied, ranges between 8 and 19%. Exceptionally low figures have been found by Harkema (1936), and particularly by Baldassari (1935), and a high incidence of 48% was reported by Frye & Meleney (1932). The two figures available for the incidence in wild mice (24·1%, Frye & Meleney, 1932; 13·09%, Harkema, 1936) suggest a similar range to that of rats.

Table 10. *Incidence of* Entamoeba muris *in wild rats*

No. examined	Incidence (%)	Geographical location	Author
472	16·3	London	Balfour, 1922
400	17·25	London	Stevenson, 1922
225	0·4	Toulon	Baldassari, 1935
110	4·4	Moscow	Epstein & Matevosjan, 1929
80	6·25	Italy	Liddo & Sangiorgi, 1941
339	18·84	Iraq	Senekji, 1940
2515	10·4	Baltimore, U.S.A.	Andrews & White, 1936
100	8·0	St Louis, U.S.A.	Tsuchiya & Rector, 1936
48	47·9	Tennessee, U.S.A.	Frye & Meleney, 1932
54	1·8	N. Carolina, U.S.A.	Harkema, 1936

strain was lost after 7 days. They were morphologically indistinguishable from the stock culture, but unfortunately no cysts were observed in the caecal contents or culture. Owing to the large volume of material inoculated relative to the size of the caecum, it is possible that the conditions inside the caecum approached that of the culture tube, thus encouraging the survival of amoebae.

Attempts were also made to infect rats and mice with *E. coli* by the oral inoculation of culture cysts. Young animals (aged 3–4 weeks) were used in Exps. 54, 56, 59, whilst older animals (12 weeks old) were used in Exps. 63 and 66. The experimental animals were examined by faecal and caecal examinations in order to determine that they were free from natural infections of *E. muris*, after which they were inoculated orally with cysts. The inoculum

Andrews & White (1936), in their extensive work, found no difference between the incidence of *E. muris* in adult and young rats, nor were they able to find any seasonal variation as was thought to occur by Stevenson (1922). Senekji (1940) reported that he observed *E. coli* as well as *E. muris* in his survey, and gave the incidence of *E. coli* infection as 0·88%, but he does not state how he differentiated the two species. Elton, Ford, Baker & Gardner (1931) found an incidence of 41, 48, and 50%, of *E. muris* in three species of wild mice, *Apodemus sylvaticus*, *Evotomys glareolus* and *Microtus hirtus* respectively.

Reports show that wild black rats (*Rattus rattus*)

are naturally parasitized by *Entamoeba muris* (Balfour, 1922; and above, p. 345), but practically all of the work on the incidence in wild rats has been carried out with *Rattus norvegicus* as this species is usually more abundant than *R. rattus*.

The incidence of *Entamoeba muris* in laboratory rats and mice, is often higher than that reported for wild animals, and this is due to the close contact of animals housed under laboratory conditions which facilitates the transference of cysts from one animal to another.

Since the incidence of *E. muris* in the laboratory murines depends upon the presence of cyst-passers among the stock, and the opportunities available for infecting fresh animals, it is not surprising that different laboratories have reported different figures. The highest result was that recorded by Ratcliffe (1929), who found that all rats aged 4–12 weeks in his stock were infected with *E. muris*. Rats may become infected at an early age; two papers published recently describing the occurrence of amoebae in rats weighing 25–30 g. (3–4 weeks old) reported that the incidence in one case was 56% (28/50 positive, Schneider & Montezin, 1948) and in the other about 50% (Fulton & Joyner, 1948). In this laboratory, on the other hand, no rats were found infected at an age of 3 weeks (fifty-nine examined). Kessel (1924) observed that the incidence of amoebae in rats increased to a maximum when the rats were aged 2–10 months, but after this age the incidence decreased.

The incidence of *E. muris* in laboratory mice also varies considerably, for instance, Kessel (1924) found that it varied from 17 to 71% in different batches of animals.

(2) *Geographical distribution*

E. muris has probably a world-wide distribution: it has been found wherever the parasitic fauna of wild rats and mice has been properly studied. In addition to the geographical locations given in Table 10, *E. muris* has been reported from wild animals found in Petrograd (Yakimoff & Miller, 1925), Italy (Vanni, 1937) and South Africa (Macgregor, 1932).

VI. HOST-RESTRICTION OF *ENTAMOEBA MURIS*

It has been shown above that a parasite, morphologically identical to *E. muris* found in the golden hamster (*Cricetus auratus*), was experimentally transferred to rats and mice. Amoebae have been reported from other species of rodents, none of which differs morphologically (where this has been studied in sufficient detail to warrant any conclusion) from *Entamoeba muris* as described above, but many of them have been named on the basis of their discovery in different hosts. Table 11 is a list of rodents from which amoebae have been recorded. The rodents are arranged according to the classification of Simpson (1945).

The amoebae found by Macgregor (1932) in *Tatera lobengulae* appears to be similar to *Entamoeba muris*, but the parasite discovered by Fantham (1926) may be a different species.

Holmes (1923) attempted to transfer cysts of the guinea-pig amoebae to two rats but was unsuccessful. Apart from this work and that reported in the present investigation no attempt has been made to determine the degree of host-restriction to the rodent hosts, nor has any attempt been made to transfer *E. muris* to any species of primate.

The infection of chicks by *E. muris* has been reported by Hegner (1929*a*,*b*). Trophic amoebae were inoculated by oral and rectal routes, but the infections obtained were transitory and the appearance of cysts often terminated the infection. The amoebae from the domestic fowl (*Entamoeba gallinarum*) as described by Tyzzer (1920), appears to be morphologically similar to *E. muris* (and *E. coli*) and may therefore be a form of these species adapted to the higher body temperature and other environmental conditions of the avian host.

VII. THE CLASSIFICATION OF *ENTAMOEBA MURIS*

The successful experimental cross-infection of rats and mice with heterologous strains of *E. muris*, substantiates the previous work of Kessel (1924), and confirms the conclusion already deduced from morphological grounds, that the amoebae found in *Rattus norvegicus* are identical with those parasitizing *Mus musculus*. The morphology of *Entamoeba muris* occurring in *Rattus rattus* is indistinguishable from that found in *R. norvegicus* and *Mus musculus*, and there is no reason to believe that the amoebae in *Rattus rattus* behave differently to those occurring in the other host species.

The morphology of the amoebae of the golden hamster (*Cricetus auratus*) described by Deviny (1941) and Neal (1947: in this paper, the hamster was erroneously described as the Syrian hamster), showed that it was extremely similar to *Entamoeba muris*. Further observations have convinced me that the hamster amoeba is indistinguishable from *E. muris*, and the ease of cross-infection to rats and mice leaves no doubt that it is identical with *E. muris*.

Since the discovery and description of *E. muris* in rats and mice, amoebae have been found in other rodents (see above); from the published description, these amoebae appear to be very similar to, if not indistinguishable from, *E. muris*. Some workers (e.g. Mackinnon & Dibb, 1938; Elton *et al.* 1931) have placed the amoebae observed in different hosts in the species *E. muris*, whilst others (Becker, 1926; Crouch,

1936; and others) have created new species only on the grounds of a new host record. The validity of these 'species' is doubtful, and it is probable that at least some of them are actually *E. muris*, but only further work on the morphology, and especially the degree of host-restriction of these parasites will show whether or not this is true.

sence of *E. muris*. Regendanz's observation that *E. coli* could be transferred to rats by rectal inoculation has not been confirmed, since the inoculation of amoebae and cysts by the intra-caecal route used above ensures that viable forms reach a site where they are likely to survive.

The results of the cross-infection experiments

Table 11. *Species of rodents recorded as hosts of amoebae morphologically similar to* Entamoeba muris

No.	Species of rodents	Species of *Entamoeba*	Observer
	Order LAGOMORPHA		
	Family Leporidae		
1	'Hare'	Not named	Rudovsky, 1923
2	*Lepus californicus c.*	*cuniculi*	Ward, 1934
3	'Rabbit'	*cuniculi*	Brug, 1918; Cheissin, 1938
4	*Sylvilagus floridanus alacer*	*cuniculi*	Ward, 1934
	Order RODENTIA		
	Family Sciuridae		
5	*Citellus fulvus*	*citelli*	Sassuchin *et al.*, 1930
6	*C. pygmaeus*	*citelli*	Sassuchin, 1931
7	*C. tridecemlineatus*	*citelli*	Becker, 1926
8	*Eutamias asiaticus*	*citelli*	Tanabe & Okinami, 1940
9	*Marmota bobac*	*bobaci*	Yuan-Po, 1928
10	*M. monax*	*marmotae*	Crouch, 1936
11	*Sciurus niger rufiventer*	Not named	Graham & Uhrich, 1943
	Family Heteromyidae		
12	*Dipodomys spectabilis*	*dipodomysi*	Hegner, 1926
	Family Cricetidae		
13	*Cricetus auratus*	Not named	Neal, 1947
14	*C. cricetus*	*criceti*	Starkoff, 1942
15	*Evotomys glareolus*	*muris*	Elton *et al.*, 1931
16	*Gerbillus meridianus*	*citelli*	Sassuchin *et al.*, 1930
17	*Lophiomys imhausi*	*muris*	Mackinnon & Dibb, 1938
18	*Microtus hirtus*	*muris*	Elton *et al.*, 1931
19	*M. orcadensis*	Not named	Fulton & Joyner, 1948
20	*Sigmodon hispidus*	Not named	Fulton & Joyner, 1948
21	*Tatera lobengulae*	Not named	Macgregor, 1932
	Family Muridae		
22	*Apodemus sylvaticus*	*muris*	Elton *et al.*, 1931
23	*Cricetomys gambianus*	*muris*	Mackinnon & Dibb, 1938
24	*Mus musculus*	*muris*	Grassi, 1879
25	*Rattus norvegicus*	*muris*	Balfour, 1922
26	*R. rattus*	*muris*	Balfour, 1922
	Family Dipodidae		
27	*Dipodipus sagitta*	*citelli*	Sassuchin, 1931
28	'Jerboa'	Not named	Wenyon, 1926
	Family Caviidae		
29	*Cavia porcellus*	*cobayae*	Chatton, 1917
	Family Hydrochoeridae		
30	*Hydrochoerus capybara*	*muris*	Mackinnon & Dibb, 1938

In the present work, a total of fifty-seven rats and mice were inoculated with cysts of various strains of *E. coli*, and only one animal developed a doubtful amoebic infection (see p. 359). These results are contrary to those given by Kessel (1924) and Regendanz (1929). However the methods used by these authors were not sufficiently exacting to eliminate the pre-

described above show that *E. coli* has become adapted to man and monkeys, and will not infect rats and mice. In spite of this difference, the host-parasite relationship of *E. muris* closely parallels that of *E. coli*, in its non-pathogenicity, resistance to treatment with drugs (Neal, 1949), the duration of viability of cysts, the course of infection which in

both cases is self-limited, and in having a similar habitat.

It has already been stressed by previous observers (Wenyon, 1907; Brug, 1919) that the morphology of *E. muris* is extremely similar to that of *E. coli*. Wenrich (1940) considers that the nuclear structure of *E. muris* is intermediate between that of *E. coli* and *E. histolytica*. As a matter of fact, however, the nuclear characters of *E. muris* differ markedly from those of *E. histolytica*, but are indistinguishable from those of *E. coli*, as described by Dobell (1919) and others. The only differences between *E. coli* and *E. muris* are that the karyosome may be more eccentric in the latter than in the former, and the mode of the smaller race of *E. muris* is smaller than that reported by Matthews (1919) for *E. coli*. On these grounds, therefore, there appears to be little justification for the separation of *E. coli* from *E. muris*, but it must be remembered that an important stage of the life-history of the latter species has not yet been elucidated, viz. the excystment and metacystic development, and a final taxonomic decision cannot be made until these have been studied, although in the light of the cross-infection work reported above, *E. muris* appears to be distinct from *E. coli*.

In conclusion, it may be stated that at the present state of our knowledge it is evident that *E. muris* differs from *E. coli* only in physiological characters, while morphologically it is similar to it. However, if future investigations reveal some difference in the metacystic development of the murine amoeba, its relation to *E. coli* will have to be revised.

VIII. SUMMARY

1. A description of the morphology and life history (excluding metacystic development) of *Entamoeba muris* is given.

2. The descriptions of species of amoebae found in rats (*Rattus norvegicus* and *R. rattus*) and mice (*Mus musculus*) by previous authors are critically discussed, and it is concluded that one species, *Entamoeba muris*, is present.

3 Experiments on cross-infection of rats and mice with *E. muris* are described, together with observations on the course of these infections.

4. The transference of an amoeba morphologically similar to *E. muris* from *Cricetus auratus* to rats and mice is described.

5. An account is given of unsuccessful attempts to establish an infection of *Entamoeba coli* in rats and mice.

6. Observations on the incidence and host-restriction of *E. muris* are discussed.

7. The affinities of *E. coli* and *E. muris* are discussed and it is concluded that, although *E. muris* resembles *E. coli* morphologically, it differs from *E. coli* on physiological grounds.

I should like to thank Dr C. A. Hoare in particular, for suggesting and directing this work through all its stages, Mr L. G. Goodwin for his criticism and developing the statistical treatment, and Dr C. Dobell, F.R.S., for his advice and for supplying some of the strains of *E. coli*. I am also indebted to Miss S. I. Pluthero and Mr D. Cox for technical assistance.

REFERENCES

ANDREWS, J. & WHITE, H. F. (1936). An epidemiological study of protozoa parasitic in wild rats in Baltimore, with especial reference to *Endamoeba histolytica*. *Amer. J. Hyg.* **24**, 184.

BALDASSARI, M. T. (1935). Le parasitisme des rats à Toulon. *Marseille méd.* **72**, 716.

BALFOUR, A. (1922). Observations on wild rats in England with reference to their ecto- and endo-parasites. *Parasitology*, **14**, 282.

BECKER, E. R. (1926). *Endamoeba citelli* sp.nov. from the striped ground squirrel *Citellus tridecemlineatus*, and the life-history of its parasite, *Sphaerita endamoebae* sp.nov. *Biol. Bull. Woods Hole*, **50**, 444.

BRUG, S. L. (1918). *Entamoeba cuniculi* n.sp. *Geneesk. Tijdschr. Ned.-Ind.* **58**, 811.

BRUG, S. L. (1919). De entamoeben van de rat. *Geneesk. Tijdschr. Ned.-Ind.* **59**, 1.

CHATTON, E. (1917). Réalisation expérimentale chez le cobaye de l'amibiase intestinale à *Entamoeba dysenteriae*. *Bull. Soc. Path. exot.* **10**, 794.

CHEISSIN, E. (1938). [Some observations on the morphology of *E. coli* forma *cuniculi* Brug from the intestine of the rabbit.] *J. Microbiol., Epidemiol., Parasitol.* **17**, 384. (Russian with English summary.)

CHIANG, S. F. (1925*a*). The rat as a possible carrier of the dysentery amoeba. *Proc. Nat. Acad. Sci., Wash.*, **11**, 239.

CHIANG, S. F. (1925*b*). Study of parasitic amoebae by experimental cross-infection of laboratory animals. *Nat. med. J. China*, **11**, 440.

CROUCH, H. B. (1936). Animal parasites of the woodchuck with reference to the protozoa. *Iowa St. Coll. J. Sci.* **11**, 48.

DEVINY, E. (1941). The protozoa of the intestine of the golden hamster *Cricetus auratus*. Unpublished thesis. Glasgow University.

DOBELL, C. (1919). *The Amoebae Living in Man*. London: John Bale, Sons and Danielsson.

DOBELL, C. (1936). Researches on the intestinal protozoa of monkeys and man. VIII. An experimental study of some simian strains of '*Entamoeba coli*'. *Parasitology*, **28**, 541.

DOBELL, C. (1942). Some new methods for studying intestinal amoebae and other protozoa. *Parasitology*, **34**, 101.

DOBELL, C. & LAIDLAW, P. P. (1926). On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. *Parasitology*. **18**, 283.

ELTON, C., FORD, E. B., BAKER, J. R. & GARDNER, A. D. (1931). The health and parasites of a wild mouse population. *Proc. zool. Soc. Lond.* p. 657.

EPSTEIN, G. W. [=EPSTEIN, H.] (1941). *Parasitic Amoebae*. Ed. by E. N. Pavlovsky. Moscow, Leningrad (in Russian).

EPSTEIN, H. [=EPSTEIN, G. W. & MATEVOSJAN, S.] (1929). Untersuchungen über Rattenamöben. *Arch. russes. Protist. Moscow*, **8**, 142. (Russian with a German summary.)

FANTHAM, H. B. (1926). Some parasitic protozoa found in South Africa. IX. *S. Afr. J. Sci.* **23**, 550.

FRYE, W. W. & MELENEY, H. E. (1932). Investigations of *Endamoeba histolytica* and other intestinal protozoa in Tennessee. IV. A study of flies, rats, mice, and some domestic animals as possible carriers of the intestinal protozoa of man in a rural community. *Amer. J. Hyg.* **16**, 729.

FULTON, J. D. & JOYNER, L. P. (1948). Natural amoebic infections in laboratory rodents. *Nature, Lond.*, **161**, 66.

GOODSON, J. A., GOODWIN, L. G., GORVIN, J. H., GOSS, M. D., KIRBY, K. S., LOCK, J. A., NEAL, R. A., SHARP, T. M. & SOLOMON, W. (1948*a*). The Chemotherapy of Amoebiasis. II. Amines derived formally from emetine. *Brit. J. Pharmacol.* **3**, 49.

GOODSON, J. A., GOODWIN, L. G., GORVIN, J. H., GOSS, M. D., KIRBY, K. S., LOCK, J. A., NEAL, R. A., SHARP, T. M., & SOLOMON, W. (1948*b*). The Chemotherapy of Amoebiasis. III. Variants of bis-(diamylamino) decane. *Brit. J. Pharmacol.* **3**, 62.

GOODWIN, L. G., HOARE, C. A. & SHARP, T. M. (1948). The Chemotherapy of Amoebiasis. Part I. Introduction and methods of biological assay. *Brit. J. Pharmacol.* **3**, 44.

GRAHAM, E. & UHRICH, J. (1943). Animal parasites of the fox squirrel *Sciurus niger rufiventer*, in Southeastern Kansas. *J. Parasit.* **29**, 159.

GRASSI, B. (1879). Dei Protozoi parassiti e specialmente di quelle che sono nell' uomo. *Gaz. med. ital. Lombard.* **39**, 445.

GRASSI, B. (1882). Intorno ad alcuni protisti endoparassitici, ed appartenenti alle classi dei Flagellati, Lobosi, Sporozoi e Ciliati. *Atti Soc. ital. Sci. nat.* **24**, 135.

HARKEMA, R. (1936). The parasites of some N. Carolina rodents. *Ecol. Monagr.* **6**, 151.

HAWES, R. S. (1947). On the structure, division, and systematic position of *Trichomonas vaginalis* Donné. with a note on its methods of feeding. *Quart. J. micr. Sci.* **88**, 79.

HEGNER, R. (1926). *Giardia beckeri* n.sp. from ground squirrel and *Endamoeba dipodomysi* n.sp. from the kangaroo rat. *J. Parasit.* **12**, 203.

HEGNER, R. (1929*a*). Transmission of intestinal protozoa from man and other animals to parasite-free fowls. *Amer. J. Hyg.* **9**, 529.

HEGNER, R. (1929*b*). The infection of parasite-free chicks with intestinal protozoa from birds and other animals. *Amer. J. Hyg.* **10**, 33.

HOLMES, F. O. (1923). Observations on the cysts of *Entamoeba cobayae*. *J. Parasit.* **10**, 47.

JONES, W. R. (1946). The experimental infection of rats with *Entamoeba histolytica*: with a method for evaluating the anti-amoebic properties of new compounds. *Ann. trop. Med. Parasit.* **40**, 130.

KESSEL, J. F. (1923). Experimental infection of rats and mice with the common intestinal amoebae of man. *Univ. Calif. Publ. Zool.* **20**, 409.

KESSEL, J. F. (1924). The distinguishing characteristics of the parasitic amoebae of culture rats and mice. *Univ. Calif. Publ. Zool.* **20**, 489.

KESSEL, J. F. (1930). Methods and problems of cross infection experiments with intestinal protozoa of man and certain laboratory animals. Chapter 10 in Hegner and Andrews, *Problems and Methods of Research in Protozoology*. New York: The Macmillan Co.

KOFOID, C. A., MCNEIL, E. & BONESTELL, A. (1933). Correlation of the distribution of protozoa in the intestine of *Rattus norvegicus* with the hydrogen ion concentration of the intestinal contents and wall. *Univ. Calif. Publ. Zool.* **39**, 179.

KOFOID, C. A. & SWEZY, O. (1921). On the free, encysted, and budding stages of *Councilmania lafleuri*, a parasitic amoeba of the human intestine. *Univ. Calif. Publ. Zool.* **20**, 169.

KUWABARA, N. (1931). On the cultivation of *Entamoeba muris* (Grassi, 1879) *in vitro*. *Keijo J. Med.* **2**, 28.

LEVITANSKAÏA, P. B. (1938). Réaction nucléique de Feulgen chez les protozoaires intestinaux. *Bull. Biol. Med. exp.* **6**, 403.

LIDDO, S. & SANGIORGI, M. (1941). [Parasitological data on the rats of Bari.] *Riv. Parasitol.* **5**, 241.

MACGREGOR, E. L. (1932). Some endoparasites of certain South African rodents. Unpublished thesis, University of the Witwatersrand.

MACKINNON, D. L. & DIBB, M. J. (1938). Report on intestinal protozoa of some mammals in the Zoological Gardens at Regent's Park. *Proc. zool. Soc. Lond.* B, **108**, 323.

MATTHEWS, J. R. (1919). A mensurative study of the cysts of *Entamoeba coli*. *Ann. trop. Med. Parasit.* **12**, 259.

MCCLUNG, C. E. (1937). *Handbook of Microbioscopical Technique*. London: Oxford University Press.

MEGLITSCH, P. A. (1939). Nucleic acid cycle in *Endamoeba blattae*. *J. Parasit.* **25**, 441.

NEAL, R. A. (1947). *Entamoeba* sp. from the Syrian Hamster (*Cricetus auratus*). *Nature, Lond.*, **159**, 502.

NEAL, R. A. (1948). *Entamoeba histolytica* in wild rats caught in London. *J. Hyg., Camb.*, **46**, 90.

NEAL, R. A. (1949). *Entamoeba muris*: a complicating factor in the experimental infection of rats with *E. histolytica*. *Nature, Lond.*, **163**, 99.

PAVLOVA, E. A. (1938). [Comparative study of locomotion in *Entamoeba histolytica* and *E. coli*.] *Med. Parasitol., Moscow*, **7**, 110. (In Russian.) Abstract in *Trop. Dis. Bull.* 1939, **36**, 284.

RATCLIFFE, H. L. (1929). A method for obtaining a breeding stock of rats free from intestinal protozoa. *Science*, **70**, 286.

REGENDANZ, P. (1929). Ueber die Uebertragung der *Entamoeba histolytica*, *Entamoeba coli*, und *Dientamoeba fragilis* auf Ratten. *Zbl. Bakt. Originale*, **111**, 412.

RUDOVSKY, F. (1921). Über Rattenamöbiase. *Wien. tierärztl. Mschr.* **8**, 189.

RUDOVSKY, F. (1923). Beobachtungen an Protozoen. *Wien. tierärztl. Mschr.* **10**, 142.

SASSUCHIN, D. N. (1931). Zum Studium der Darmprotozoenfauna der Nager im Süd-Osten RSFSR. 1. Darmprotozoen des *Citellus pygmaeus* Pallas. *Arch. Protistenk.* **74**, 417.

SASSUCHIN, D. N., POPOFF, P. P., KUDRJEWZEW, W. A. & BOGENKO, W. P. (1930). Über parasitische infektion bei Darmprotozoen. *Arch. Protistenk.* **71**, 229.

SCHNEIDER, J. & MONTEZIN, G. (1948). Amibiase expérimentale et amibiase spontanée du rat. *Bull. Soc. Path. exot.* **41**, 521.

SENEKJI, H. A. (1940). Bacteriological and parasitological survey of rats in Iraq. *Trans. R. Soc. trop. Med. Hyg.* **33**, 655.

SIMPSON, G. G. (1945). The principles of classification and a classification of mammals. *Bull. Amer. Mus. nat. Hist.* **85**, 1.

SMITH, S. (1928). Host-parasite relationships of *Iodomoeba williamsi* in guinea-pigs and rats. *Amer. J. Hyg.* **8**, 1.

STABLER, R. M. (1932). An extended study of variations in a single race of coli-like amoebae and its bearing on the specificity of *Councilmania lafleuri*. *Amer. J. Hyg.* **16**, 1.

STARKOFF, O. (1942). *Entamoeba criceti* n.sp., parassita del *Cricetus cricetus*. *Riv. Parassitol.* **6**, 57.

STEVENSON, A. C. (1922). The incidence of a Leptospira in the kidneys and of parasites in the intestines of one hundred wild rats examined in England. *Amer. J. trop. Med.* **2**, 77.

TANABE, M. & OKINAMI, M. (1940). On the parasitic protozoa of the ground squirrel *Eutamias asiaticus* Uthensis, with special reference to *Sarcocystis eutamias* sp. nov. *Keizyô J. Med.* **10**, 126.

TSUCHIYA, H. & RECTOR, L. E. (1936). Studies on intestinal parasites among wild rats caught in St Louis. *Amer. J. trop. Med.* **16**, 705.

TYZZER, E. E. (1920). Amoebae of the caeca of the common fowl and of the turkey—*Entamoeba gallinarum* n.sp. and *Pygolimax gregariniformis*, gen. et spec.nov. *J. med. Res.* **41**, 199.

VANNI, V. (1937). Ricerche parassitologiche sui ratti di Roma. *Ann. Igiene*, **47**, 509.

WANG, C. C. & NIE, D. (1934). Notes on *Entamoeba muris* (Grassi) and *Trichomonas caviae* Devaine. *Proc. Fifth Pan-Pacific Sci. Congress*, **4**, 2991.

WARD, J. W. (1934). A study of some parasites of Central Oklahoma. *The Biologist*, **15**, 83. From Harkema (1936).

WENRICH, D. H. (1940). Nuclear structure and nuclear division in the trophic stages of *Entamoeba muris* (Protozoa, Sarcodina). *J. Morph.* **66**, 215.

WENRICH, D. H. (1941*a*). Observations on the food habits of *E. muris* and *E. ranarum*. *Biol. Bull. Woods Hole*, **81**, 324.

WENRICH, D. H. (1941*b*). The morphology of some protozoan parasites in relation to microtechnique. *J. Parasit.* **27**, 1.

WENYON, C. M. (1907). Observations on the protozoa in the intestine of mice. *Arch. Protistenk.* Suppl. **1**, 169.

WENYON, C. M. (1925). The genera *Councilmania*, *Karyamoeba*, and *Caudamoeba*. *Trop. Dis. Bull.* **22**, 333.

WENYON, C. M. (1926). *Protozoology*, p. 229. London: Baillière, Tindall and Cox.

WIGHT, T. H. J. & PRINCE, L. H. (1927). Artefacts in entamoebae which have led to the naming of a new genus and species. *Amer. J. trop. Med.* **7**, 287.

YAKIMOFF, W. L. & MILLER, G. A. (1925). Les protozaires de l'intestin des rats d'égout. *Bull. Soc. Path. exot.* **18**, 311.

YUAN-PO, L. (1928). *Entamoeba bobaci* n.sp. des tarabagons (*Marmota bobac*). *Ann. Parasit.* **6**, 339.

(*MS. received for publication* 3. XI. 1949.—Ed.)

THE MITES (ACARINA) PARASITIC ON MAMMALS, BIRDS AND REPTILES

By CHARLES D. RADFORD

Membre Correspondant, Muséum National d'Histoire Naturelle, Paris

INTRODUCTION

The author (1943) published a paper under the title 'Genera and Species of Parasitic Mites (Acarina)'. This paper has been in great demand by workers interested in the parasitic Acarina and the number of separates was speedily exhausted.

With the impetus given to Acarological studies during the war years (1939–45) a considerable amount of work has been done on the parasitic mites, most of which, as parasites of mammals, birds and reptiles are potential—a few are acknowledged—disease carriers.

The original paper had a number of shortcomings—for instance, the genera were not arranged systematically—and there were omissions. A number of additional genera and species have been added to make the present paper up to date and the genera have been arranged systematically in accordance with the author's paper 'Systematic Check List of Mite Genera and Type Species' (in the Press).

The subject of ticks (Ixodoidea) and disease transmission is considered to be a separate, specialized study, and for this reason the Ixodoidea, although in the order Acarina, are excluded from this paper. The mites of the family Trombiculidae, many of the genera of which are founded on larval characters, although parasitic in their larval stages on mammals, birds and reptiles are excluded from this paper as they are considered a large and important group to which full justice could be given only in a separate work. The feather mites (Analgesidae) are also excluded for, although they are found on birds and are mainly host specific, they are not injurious to their hosts, living upon the broken quill tissues only.

I wish to acknowledge the assistance given in the compilation of the Host List by Mr H. W. Parker, Keeper, Department of Zoology, British Museum (Nat. Hist.), London, and members of his department.

Order **ACARINA** Nitzsch, 1818

Family LAELAPTIDAE Berlese, 1892

Subfamily HYPOASPIDINAE Vitzthum, 1941

Genus **Androlaelaps** Berlese, 1903

A. arvicanthis Radford, 1944	*Arvicanthis abyssinicus rubescens* Wroughton, 1909
Type: *A. hemaphrodita* (Berlese, 1887)	
A. karawaiewi Berlese, 1903	*Formica pratensis*
A. marshalli Berlese, 1911	Rats
A. oudemansi Radford, 1944	*Arvicanthis abyssinicus rubescens* Wroughton, 1909
A. sardous Berlese, 1911	*Apodemus sylvaticus sylvaticus* L. 1758
A. setosus Fox, 1947	*Rattus norvegicus norvegicus* Berkenhout, 1769

Genus **Haemolaelaps** Berlese, 1910

H. calosus Berlese, 1917	*Lophuromys aquilus zena* Dollman, 1892
H. capensis Hirst, 1916	*Cryptomys hottentotus hottentotus* Lesson, 1826
H. centrocarpus Berlese, 1911	Rats
H. concurrens Berlese, 1918	*Muscis*
H. elongatus Berlese, 1918	Detritus
H. fahrenholzi Berlese, 1911	*Apodemus sylvaticus sylvaticus* L. 1758
H. inops Berlese, 1911	Rats
H. inops var. *zulu* Berlese, 1918	*Lemniscomys griselda spinalis* Thomas, 1916
H. inversus Berlese, 1918	Detritus
Type: *H. marsupialis* Berlese, 1910	*Perameles* sp.
H. mauritanicus Hirst, 1925	*Gerbillus hirtipes* Lataste, 1882
H. mesopicos Radford, 1942	*Mesopicos ruwenzorii* Sharpe, 1902

H. mohrae Oudemans, 1925	*Microtus arvalis arvalis* Pallas, 1778
H. molestus Oudemans, 1929	
H. murinus Berlese, 1911	Rats
H. mystromys Radford, 1942	*Mystromys albicaudatus albicaudatus* Smith, 1834
H. omnitectus Vitzthum, 1928	*Chrysocolaptes validus* Temminck
H. phialiger Berlese, 1917	*Acromyrmex lundi*
H. scalopi Keegan, 1946	*Scalopus aquaticus* (L. 1758)
H. scapularis Berlese, 1918	*Spalax microphthalmus* Guldenstaedt, 1770
H. spinitarsus Berlese, 1918	*Georychus* sp.
H. spinosulus Berlese, 1921	
H. subterraneus Berlese, 1921	
H. tachyoryctes Radford, 1941	*Tachyoryctes ruddi ruddi* Thomas, 1909
H. villosissimus Berlese, 1918	*Saccostomus campestris campestris* Peters, 1846

Subfamily HALARACHNINAE Oudemans, 1906

Genus **Halarachne** Allman, 1847

(Syn. *Rhinixodes*; *Rhinacarus* Nehring, 1884)

H. americana Banks, 1899	*Monachus tropicalis* (Gray, 1850)
Type: *H. halichoeri* Allman, 1847	*Halichoerus grypus* (Fabricius, 1791)
H. miroungae Ferris, 1925	*Mirounga angustirostris* (Gill, 1866)
Halarachne sp. Blanchard, 1906	*Leptonychotes weddelli* (Lesson, 1826)

Genus **Orthohalarachne** Newell, 1947

Type: *O. attenuata* (Banks, 1910)	Seal pup
O. diminuata (Doetschman, 1944)	*Zalophus californianus* (Lesson, 1866)
O. magellanica (Finnegan, 1934)	*Otaria byronia* (de Blainville, 1820)
O. reflexa (Tubb, 1937)	Tasmanian sea bear
O. rosmari (Oudemans, 1916)	*Odobenus rosmarus* (L. 1758)
O. zalophi (Oudemans, 1916)	*Zalophus californianus* (Lesson, 1866)

Syn. *H. otariae* Steding, 1923

Genus **Pneumonyssus** Banks, 1901

P. caninum Chandler & Ruhe, 1940	*Canis familiaris* L. 1766
P. congoensis Ewing, 1929	*Papio porcarius* Boddaert, 1787
P. dinolti Oudemans, 1935	*Macaca mulatta* Zimmerman, 1780
P. duttoni Newstead & Todd, 1906	*Cercopithecus schmidti* Matschie, 1892
P. procavians Radford, 1938	*Procavia capensis* Pallas, 1766
Type: *P. simicola* Banks, 1901	*Macaca nemestrina* L. 1766
P. stammeri Vitzthum, 1930	*Lagothrix infumatus* Spix, 1823

Subfamily HAEMOGAMASINAE Oudemans, 1926

Genus **Haemogamasus** Berlese, 1889

H. alaskensis Ewing, 1925	*Microtus* sp.
H. avisugus Vitzthum, 1930	*Riparia riparia* (L. 1758)
H. barberi Ewing, 1925	
Type: *H. hirsutus* Berlese, 1889	*Talpa europaea* L. 1758
H. horridus var. *arvicolarum* Berlese, 1920	*Microtus arvalis arvalis* Pallas, 1778
H. liberiensis Hirst, 1916	*Hybomys trivirgatus trivirgatus* Temminck, 1853
H. liponyssoides Ewing, 1925	*Scalops argentatus* Andubon & Bachmann, 1842
H. mandschuricus Vitzthum, 1930	*Phodopus roborovskii bedfordiae* Thomas, 1908
H. microti Ewing, 1925	*Microtus* sp.
H. reidi Ewing, 1925	*Sciurus* sp.
H. sanguineus Ewing & Stover, 1915	*Rattus rattus rattus*, L. 1758
H. sternalis Ewing, 1933	*Blarina brevicauda talpoides* Gapper, 1830
H. twitchelli Ewing, 1925	*Clethrionomys dawsoni dawsoni* Merriam, 1888

Genus **Euhaemogamasus** Ewing, 1933

E. horridus (Michael, 1892)	*Apodemus sylvaticus sylvaticus* L. 1758
E. nidi (Michael, 1892)	*Talpa europaea* L. 1758
Type: *E. onychomydis* Ewing, 1933	*Onychomys* sp.
E. oregonensis Ewing, 1933	*Phenacomys albipes* Merriam, 1901
E. oudemansi (Hirst, 1914)	*Rattus norvegicus norvegicus* Berkenhout, 1769
E. quadrisetatus (Vitzthum, 1926)	*Akodon olivaceus* Waterhouse, 1837
E. sciuropteri Keegan, 1946	*Sciurus* sp.
E. utahensis Ewing, 1933	*Neotoma lepida lepida* Thomas, 1893
Type: *E. hollisteri* (Ewing, 1925)	*Peromyscus californicus californicus* Gambel, 1848
E. rotundus Fonseca, 1935	Wild rat

Genus **Geneiadolaelaps** Ewing, 1929

Type: *G. barbatus* (Ewing, 1925)	Lemur
G. spenceri Gunther, 1942	Man

Genus **Cavilaelaps** Fonseca, 1935

Type: *C. breslaui* Fonseca, 1935	*Caviella australis australis* Geoffroy & D'Orbigny, 1833

Genus **Neoparalaelaps** Fonseca, 1935

Syn. *Paralaelaps* Fonseca, 1935

Type: *N. bispinosus* (Fonseca, 1935)	*Cavia aperea aperea* Erxleben, 1777

Genus **Echinolaelaps** Ewing, 1929

Type: *E. echidninus* (Berlese, 1887)	*Rattus norvegicus norvegicus* Berkenhout, 1769

Genus **Gigantolaelaps** Fonseca, 1938

G. brachyspinosus (Fonseca, 1935)	*Holochilus vulpinus* Lichtenstein, 1827
G. butantanensis (Fonseca, 1935)	*Oryzomys eliurus* Wagner, 1845
G. comatus Fonseca, 1938	Field rat
G. gilmorei Fonseca, 1938	Rats
G. goyanensis Fonseca, 1938	*Estrimys* sp.
G. mattogrossensis (Fonseca, 1935)	*Holochilus vulpinus* Lichtenstein, 1827
G. maximus (Berlese, 1903)	*Oryzomys subflavus* Wagner, 1842
G. oudemansi Fonseca, 1938	Wild rat
G. peruvianus (Ewing, 1933)	Wild rat
G. versteegi (Oudemans, 1904)	*Mus* sp.
Type: *G. vitzthumi* Fonseca, 1938	Wild rat

Genus **Ugandolaelaps** Radford, 1942

Type: *U. protoxera* Radford, 1942	*Protoxerus stangeri centricola* Thomas, 1906

Genus **Acanthochela** Ewing, 1933

Type: *A. chilensis* Ewing, 1933	*Didelphys elegans*

Subfamily Laelaptinae Tragardh, 1908

Genus **Laelaps** Koch, 1842

L. aethiopicus Hirst, 1925	Rats
L. algericus Hirst, 1925	*Mus musculus algirus* Pomel, 1856
L. aragonensis Fonseca, 1938	Rats
L. arvicolae Oudemans, 1916	*Microtus arvalis arvalis* Pallas, 1778
L. berlesei Fonseca, 1938	*Galictis vittata* Schreber, 1795
L. bermudaensis (Ewing, 1922)	Wood rat
L. braziliensis Ewing, 1925	*Galea spixii* Wagler, 1831
L. buxtoni Radford, 1941	Gerbille
L. californicus Ewing, 1925	
L. cancer Pearse, 1929	*Gecarcinus lateralis* Freminville
L. differens Fonseca, 1935	Wild rat
L. exceptionalis Fonseca, 1935	Wild rat

L. finlaysoni Womersley, 1937	*Gyomys apodemoides* Finlayson, 1932
L. glasgowi Ewing, 1925	Wild rat
L. hapoloti Hirst, 1931	*Hapolotis* sp.
L. hilaroides Oudemans, 1928	*Eptesicus serotinus* Schreber, 1774
L. hirsti Fonseca, 1938	*Oryzomys eliurus* Wagner, 1845
L. jettmari Vitzthum, 1930	*Apodemus agrarius agrarius* Pallas, 1778
L. lamborni Hirst, 1925	Rats
L. lativentralis Fonseca, 1935	Wild rat
L. lemni Grube, 1851	*Lemmus obensis obensis* Brants, 1827
L. liberiensis Hirst, 1925	*Dephomys defua* Miller, 1900
L. longiventris Meillon & Lavoipierre, 1944	*Mysorex* sp.
L. manquinhosi Fonseca, 1935	*Holochilus vulpinus* Lichtenstein, 1827
L. mazzi Fonseca, 1938	Rats
L. melomys Womersley, 1937	*Melomys cervinipes littoralis* Lonneberg, 1916
L. microti (Ewing, 1933)	*Microtus californicus californicus* Peale, 1848
L. multispinosus Banks, 1909	*Ondatra zibethica zibethica* L. 1766
Type: *L. muris* (Ljungh, 1799)	*Arvicola amphibius amphibius* L. 1758
	Syn. *Laelaps agilis* Koch, 1836
L. navasi Fonseca, 1938	*Oryzomys eliurus* Wagner, 1845
L. nuttalli Hirst, 1915	*Rattus rattus rattus* L. 1758
L. oraniensis Hirst, 1925	Field mice
L. pachypus Koch, 1839	*Microtus arvalis arvalis* Pallas, 1778
L. pallidus Tragardh, 1931	*Rattus rattus rattus* L. 1758
L. parvulus Hirst, 1933	*Lemniscomys griselda spinalis* Thomas, 1916
L. paulistanensis Fonseca, 1935	White rat
L. robustipes Ewing, 1925	Rodent
L. rothschildi Hirst, 1914	*Melomys rufescens rufescens* Alston, 1877
L. soricis Oudemans, 1925	*Sorex* sp.
L. stegemani Hefley, 1935	*Mephitis nigra* Peale & Beauvois, 1796
L. thori Fonseca, 1938	
L. vansomereni Hirst, 1933	*Aethomys chrysophilus chrysophilus* de Winton, 1896
L. virginianus Ewing, 1925	Wild mouse
L. wetmorei Ewing, 1925	Rat
L. wolffsohni Oudemans, 1910	

Genus **Tricholaelaps** Vitzthum, 1926

Type: *T. comatus* Vitzthum, 1926	*Rattus whiteheadi whiteheadi* Thomas, 1894

Genus **Heterolaelaps** Hirst, 1926

Type: *H. antipodianum* Hirst, 1926	*Perameles nasuta* Geoffroy, 1804

Genus **Mesolaelaps** Hirst, 1926

Type: *M. anomalus* Hirst, 1926	*Perameles* sp.
M. australiensis Hirst, 1926	Mice
M. lagotisinus Hirst, 1931	*Thalacomys lagotis* Reid, 1837

Genus **Cyclolaelaps** Ewing, 1933

Type: *C. circularis* Ewing, 1933	*Peromyscus truei truei* Shufeldt, 1886
C. lophuromius Radford, 1939	*Lophuromys aquilus aquilus* True, 1892

Genus **Macrolaelaps** Ewing, 1929

M. bakeri (Hirst, 1923)	*Rhabdomys pumilio pumilio* Sparrmann, 1784
M. giganteus (Berlese, 1918)	*Lemniscomys griselda spinalis* Thomas, 1916
M. grandis (Hirst, 1923)	Rat
M. muricola (Tragardh, 1908)	*Mastomys coucha hildebrandti* Peters, 1878
Type: *M. sanguisugus* (Vitzthum, 1926)	*Akodon olivaceus* Waterhouse, 1837
M. sculpturatus (Vitzthum, 1926)	*Rattus whiteheadi whiteheadi* Thomas, 1894
M. ugandanus (Hirst, 1923)	Rodent

Genus **Neolaelaps** Hirst, 1926

N. echinus (Oudemans, 1925)	Bat
Type: *N. magnistigmatus* (Vitzthum, 1918)	*Pteropus edulis* Geoffroy, 1810

Genus **Longolaelaps** Vitzthum, 1926

Type: *L. longulus* Vitzthum, 1926	*Rattus whiteheadi whiteheadi* Thomas, 1894

Genus **Eulaelaps** Berlese, 1903

E. citellus Radford, 1942	*Citellus beecheyi beecheyi* Richardson, 1829
E. cricetuli Vitzthum, 1930	*Phodopus roborovskii bedfordiae* Thomas, 1908
E. grahamensis Tragardh, 1907	
E. mawsoni Womersley, 1937	
E. novus Vitzthum, 1924	*Riparia riparia* (L. 1758)
E. oribatoides (Michael, 1892)	*Talpa europaea* L. 1758
E. pedalis (Banks, 1909)	Chipmunk
E. pontiger (Berlese, 1903)	Rat
E. propheticus (Banks, 1909)	Groundhog
E. roosevelti Wharton, 1941	Boobies nest
Type: *E. stabularis* (Koch, 1836)	*Talpa europaea* L. 1758

Genus **Mysolaelaps** Fonseca, 1935

M. microspinosus Fonseca, 1935	Rat
Type: *M. parvispinosus* Fonseca, 1935	Rat

Genus **Atricholaelaps** Ewing, 1929

A. arvicanthis (Radford, 1939)	*Arvicanthis abyssinicus rubescens* Wroughton, 1909
A. bathyergus (Radford, 1939)	*Bathyergus suillus suillus* Schreber, 1782
A. chinchillulae Strandtmann, 1948	*Chinchillula sahamae* Thomas, 1898
A. coelogenys (Fonseca, 1935)	*Cuniculus paca paca* L. 1766
A. cryptomius (Radford, 1939)	*Georychus capensis capensis* Pallas, 1779
A. dasymys (Radford, 1939)	*Dasymys incomtus helukus* Heller, 1910
A. megaventralis Strandtmann, 1947	*Sigmodon hispidus texianus* Audubon & Bachman, 1853
Type: *A. reithrodontis* (Ewing, 1925)	*Reithrodon cuniculoides cuniculoides* Waterhouse, 1837
A. reticulatus (Fonseca, 1935)	*Euryzygomatomys spinosus catellus* Thomas, 1916
A. rhabdomys (Radford, 1939)	*Rhabdomys pumilio pumilio* Sparrmann, 1784
A. sciureus (Fonseca, 1935)	*Sciureus aestuans aestuans* L. 1766
A. tateronis (Radford, 1939)	*Tatera benvenuta benvenuta* Hinton & Kershaw, 1920
A. traubi Strandtmann, 1948	*Callosciurus quinquistriatus quinquistriatus* Anderson, 1871

Genus **Eubrachylaelaps** Ewing, 1929

E. crowei Jameson, 1947	*Onychomys leucogaster arcticeps* Rhoads, 1898
Type: *E. hollisteri* (Ewing, 1925)	*Peromyscus californicus californicus* Gambel, 1848
E. rotundus Fonseca, 1935	Rat

Genus **Ligialaelaps** Radford, 1942

Type: *L. ewingi* (Pearse, 1930)	*Ligia exotica* Roux

Genus **Mungosicola** Radford, 1942

Type: *M. ugandae* Radford, 1942	*Mungos mungo macrurus* Thomas

Genus **Spinolaelaps** Radford, 1940

Type: *S. jacksoni* Radford, 1940	*Rhinolophus lobatus* Peters, 1867

Genus **Bolivilaelaps** Fonseca, 1940

Type: *B. tricholabiatus* Fonseca, 1940	*Isothrix bistriatus bistriatus* Wagner, 1845

Genus **Pililaelaps** Radford, 1947

Syn. *Banksia* Radford, 1942

Type: *P. longiseta* Banks, 1909	Carrion beetle

Genus **Ophidilaelaps** Radford, 1947

Type: *O. imphalensis* Radford, 1947	*Elaphe radiata* (Schlegel, 1837)
O. piger (Berlese, 1918)	Snake

Genus **Ellsworthia** Turk, 1945

Syn. *Hemilaelaps* Ewing, 1933

Type: *E. americanus* (Ewing, 1933)	*Drymarchon corais melanurus* Dum. & Bibr. 1854
E. distinctus (Ewing, 1933)	*Elaphe obsoleta obsoleta* (Say, 1823)

Genus **Manitherionyssus** Vitzthum, 1925

Syn. *Manisicola* Lawrence, 1939

M. africana (Lawrence, 1939)	*Smutsia temmincki* Smuts, 1832
Type: *M. heterotarsus* (Vitzthum, 1925)	*S. temmincki* Smuts, 1832

Genus **Myonyssus** Tiraboschi, 1904

Type: *M. decumani* Tiraboschi, 1904	*Rattus norvegicus norvegicus* Berkenhout, 1769
M. eidmanni Sellnick, 1941	*Crematogaster impressa*
M. gigas Oudemans, 1912	Mole nest
M. jamesoni Baker, 1947	*Blarina brevicaudata* (Say)
M. minor Sellnick, 1941	

Genus **Myonyssoides** Hirst, 1925

Type: *M. capensis* Hirst, 1925	*Cryptomys hottentotus hottentotus* Lesson, 1826
M. spinosus Meillon & Lavoipierre, 1944	*Amblysommus hottentotus*

Family PNEUMOPHIONYSSIDAE Fonseca, 1940

Genus **Pneumophionyssus** Fonseca, 1940

Type: *P. aristoterisi* Fonseca, 1940	*Erythrolamprus aesculapii* (L. 1758)

Family DASYPONYSSIDAE Fonseca, 1940

Genus **Dasyponyssus** Fonseca, 1940

Type: *D. neivai* Fonseca, 1940	*Euphractus sexcinctus* L. 1758

Family DERMANYSSIDAE

Subfamily DERMANYSSINAE Kolenati, 1859

Genus **Dermanyssus** Duges, 1834

D. aegyptius Hirst, 1913	*Acomys cahirinus* Desmarest, 1819
D. americanus Ewing, 1922	*Passer domesticus* (L. 1758)
D. avium Duges, 1834	
D. brasiliensis Fonseca, 1935	*Holochilus sciureus* Wagner, 1842
D. brevis Ewing, 1936	*Otocoris alpestris merrilli*
D. chelidonis Oudemans, 1939	*Delichon urbica urbica* (L. 1758)
D. evotomydis Ewing, 1933	*Evotomys* sp.
Type: *D. gallinae* (Redi, 1674)	*Gallus domesticus* L. 1758
D. hirundinis (Herman, 1804)	*Hirundo rustica* L. 1758
D. murinus Oudemans, 1929	*Rattus rattus* var.
D. muris Hirst, 1913	*R. rattus rattus* L. 1758
D. oti Ewing, 1925	*Otus asio asio* (L. 1758)
D. prognephilus Ewing, 1933	*Progne subis subis* (L. 1758)
D. quintus Vitzthum, 1920	*Dendrocopus major* (L. 1758)
D. scutatus Ewing, 1936	*Colaptes cafer* (Gmelin, 1790)

Genus **Allodermanyssus** Ewing, 1922

Type: *A. sanguineus* (Hirst, 1914)	*Rattus rattus rattus* L. 1758

Family MACRONYSSIDAE Oudemans, 1936

Subfamily LIPONYSSINAE Ewing, 1923

Genus **Bdellonyssus** Fonseca, 1941

B. aethiopicus (Hirst, 1921)	*Macroscelides proboscideus* (Shaw, 1800)
Type: *B. bacoti* (Hirst, 1913)	*Rattus norvegicus norvegicus* Berkenhout, 1769
B. brasiliensis (Fonseca, 1939)	*Cavia aperea aperea* Erxleben, 1777
B. bursa (Berlese, 1888)	*Gallus domesticus* L. 1758
B. dendropicos (Radford, 1942)	*Dendropicus fuscescens laepidus* Cabani & Heine, 1863
B. eruditus (Fonseca, 1935)	Found on laboratory table
B. haematophagus (Fonseca, 1935)	*Eumops abrassus abrassus* (Temminck, 1827)
B. hirsti (Fonseca, 1935)	*Caviella australis australis* Geoffroy & D'Orbigny, 1833
B. lutzi (Fonseca, 1941)	Rat
B. monteiroi (Fonseca, 1941)	*Zygodontomys lasiurus* Lund, 1841
B. nagayoi (Yamada, 1941)	Man and rat
B. venezolanus (Vitzthum, 1941)	*Molossus nasutus* Spix, 1823
B. vitzthumi (Fonseca, 1941)	Wild rat

Genus **Chiroptonyssus** Augustson, 1945

Type: *C. robustipes* (Ewing, 1925)	*Tadarida mexicana* Saussure, 1860
Syn. *C. texensis* Augustson, 1945	
Liponyssus nyctinomius Radford, 1938	

Genus **Echinonyssus** Hirst, 1925

Type: *E. nasutus* Hirst, 1925	*Tupaia picta* Thomas, 1892

Genus **Hirstesia** Fonseca, 1948

Type: *H. sternalis* (Hirst, 1921)	Bat

Genus **Hirstionyssus** Fonseca, 1948

Type: *H. arcuatus* (Koch, 1839)	*Nyctalus noctula* Schreber, 1774
H. blanchardi (Trouessart, 1904)	*Marmota* (*Arctomys*) *marmota* (L. 1758)
H. butantanensis (Fonseca, 1932)	*Mus musculus albinus*
H. carnifex (Koch, 1839)	*Vespertilio serotinus* Daubenton
H. chilensis (Ewing, 1925)	*Tadarida brasiliensis* Geoffroy, 1824
H. confucianus (Hirst, 1921)	*Myospalax cansus cansus* Lyon, 1907
Syn. *Liponyssus berlesei* Hirst, 1921	
H. creightoni (Hirst, 1912)	Porcupine
H. cynomys (Radford, 1941)	*Cynomys* sp.
H. dentipes (Strandtmann, 1947)	*Sigmodon hispidus texianus* Audubon & Bachman, 1853
H. geomydis (Keegan, 1946)	*Geomys bursarius bursarius* Shaw, 1800
H. hirsti Fonseca, 1948	
H. isabellinus (Oudemans, 1913)	*Putorius* sp.
H. latiscutatus (Meillon & Lavoipierre, 1944)	*Rattus rattus rattus* L. 1758
H. liberiensis (Hirst, 1912)	Squirrel
H. macedonicus (Hirst, 1921)	*Spalax monticola thermaicus* Hinton, 1920
H. orcadensis (Turk, 1945)	*Microtus orcadensis westrae* Miller, 1908
H. otomys (Radford, 1942)	*Otomys* sp.
H. sciurinus (Hirst, 1921)	Squirrel
H. soricis (Turk, 1945)	*Sorex minutus* L. 1758

Genus **Ichoronyssus** Kolenati, 1858

I. brevipes (Ewing, 1925)	*Piranga erythromelas* Vieillot
I. britanicus (Radford, 1941)	*Nyctalus noctula* Schreber, 1774
I. constrictus (Ewing, 1923)	Wood thrush
I. crosbyi (Ewing & Stover, 1915)	*Myotis subulatus subulatus* (Say, 1823)
I. diversipilis (Vitzthum, 1920)	*Pipistrellus pipistrellus* (Schreber, 1774)

I. hasei Vitzthum, 1931	*Myotis nigricans* Wiedemann, 1876
I. hermanni Fonseca, 1948	*Molossus nasutus* Spix, 1823
I. kochi Fonseca, 1948	*Artibeus jamaicensis lituratus* Lichtenstein
I. kolenatii Fonseca, 1948	*Myotis ruber* (Geoffroy, 1806)
I. lepidopeltis (Kolenati, 1858)	*Rhinopoma microphyllum* (Brunnich, 1782)
I. mohrae Vitzthum, 1931	*Myotis nattereri* Kuhl, 1819
Type: *I. scutatus* Kolenati, 1858	*Rhinolophus ferrum equinum* Schreber, 1774
I. semitectus (Koch, 1879)	
I. spinosus Oudemans, 1902	*Myotis murinus* L. 1758
I. sternalis Ewing, 1923	Woodchuck

Genus **Kolenationyssus** Fonseca, 1948

Type: *K. athleticus* Fonseca, 1948	*Desmodus rotundus rotundus* (Geoffroy, 1810)

Genus **Fonsecaonyssus** Radford, 1950

Syn. *Leiognathus* Canestrini, 1885

Type: *F. sylviarum* (Canestrini & Fanzago, 1877)	*Sylvia atricapilla* (L. 1758)

Genus **Lepronyssoides** Fonseca, 1941

Type: *L. pereirai* (Fonseca, 1935)	Wild rat

Genus **Lepronyssus** Kolenati, 1858

Type: *L. leprosus* (Kolenati, 1857)	*Rhinolophus clivosus* Cretzschmar, 1826

Genus **Liponyssus** Kolenati, 1858

Type: *L. setosus* (Kolenati, 1856)	*Rhinolophus euryale* Blasius, 1853

Genus **Liponysella** Hirst, 1925

Type: *L. madagascariensis* (Hirst, 1921)	*Lemur mongoz albifrons* Geoffroy, 1796

Genus **Macronyssus** Kolenati, 1858

Type: *M. longimanus* (Kolenati, 1856)	*Rousettus aegyptiaca* (Geoffroy, 1810)

Genus **Neoichoronyssus** Fonseca, 1941

Type: *N. wernecki* (Fonseca, 1935)	*Didelphys marsupialis marsupialis* L. 1758

Genus **Neoliponyssus** Ewing, 1929

Type: *N. gordonensis* (Hirst, 1923)	*Mabuia quinquetaeniatus* Lichtenstein, 1823
N. lacertinus (Berlese, 1892)	*Lacerta viridis viridis* Laur. 1768
N. saurarum (Oudemans, 1901)	*L. agilis agilis* L. 1758

Genus **Ophionyssus** Megnin, 1844

O. easti (Ewing, 1925)	*Sceloporus graciosus graciosus* (Baird & Girard, 1852)
Type: *O. natricis* (Gervais, 1844)	*Coluber viridiflavus viridiflavus* Lacépède, 1789

Syn. *Ichoronyssus serpentium* Hirst, 1915

Genus **Radfordiella** Fonseca, 1948

Type: *R. oudemansi* Fonseca, 1948	*Desmodus rotundus rotundus* (Geoffroy, 1810)

Genus **Steatonyssus** Kolenati, 1858

S. arabicus (Hirst, 1921)	*Agama adramitana*
S. biscutatus (Hirst, 1921)	*Dendropicus cardinalis* (Gmelin, 1788)
S. ceratognathus (Ewing, 1923)	*Nycticeius humeralis* Rafinesque, 1818
S. cyclaspis (Oudemans, 1906)	*Pipistrellus pipistrellus* (Schreber, 1774)

Syn. *Liponyssus pipistrelli* Oudemans, 1906

S. javensis (Oudemans, 1914)	Bat
S. joaquimi (Fonseca, 1935)	*Glossophaga soricina* (Pallas, 1766)
S. murinus (Lucas, 1840)	*Myotis murinus* L. 1758

Syn. *Dermanyssus vespertilionis* Duges, 1834; *D. avium* Wagner, 1841; *Steatonyssus periblepharus* Kolenati, 1858; *S. brachypeltis* Kolenati, 1858

Type: *S. musculi* (Schrank, 1803)	*Mus musculus musculus* L. 1758
S. nyassae (Hirst, 1921)	*Macroscelides proboscideus* (Shaw, 1800)
S. occidentalis (Ewing, 1933)	*Myotis* sp.
S. spinosus Willmann, 1936	*Solenodon paradoxus* Brdt. 1833
S. sudanensis (Hirst, 1926)	*Liponycteris nudiventris* (Cretzschmar, 1826)
S. viator (Hirst, 1921)	*Cypselus affinis* Hardwick, 1831

Family ENTONYSSIDAE Fonseca, 1934

Subfamily ENTONYSSINAE Ewing, 1922

Genus **Entonyssus** Ewing, 1922

E. ewingi Hubbard, 1939	*Crotalus atrox* (Baird & Girard, 1853)
E. fragilis Keegan, 1946	*Lampropeltis getulus getulus* (L. 1766)
E. glasmacheri Vitzthum, 1935	*Elaphe quadrivittatus quadrivittatus* Holbrook, 1836
Type: *E. halli* Ewing, 1922	*Pithuophis melanoleucus* (Daudin, 1803)
E. heterodontus Keegan, 1943	*Heterodon contortrix contortrix* (L. 1766)
E. rileyi Ewing, 1924	*Crotalus durissus durissus* L. 1758
E. vitzthumi Schmidt, 1940	*Pithuopis sayi sayi* (Schlegel, 1837)

Genus **Ophiopneumicola** Hubbard, 1938

O. americana Turk, 1947	*Elaphe guttata* (L. 1766)
Type: *O. colubri* Hubbard, 1938	*Coluber flagellum flavigularis* (Hallowell, 1852)
O. elaphes Keegan, 1943	*Elaphe obsoleta obsoleta* (Say, 1823)
O. hamertoni (Radford, 1939)	*Thamnophis sirtalis sirtalis* (L. 1758)
O. natricis Keegan, 1943	*Natrix sipedon pictiventris* Cope, 1895

Genus **Hamertonia** Turk, 1947

Syn. *Hammertoni* Turk, 1947

Type: *H. bedfordi* (Radford, 1937)	*Dendroaspis angusticeps* (Smith, 1849)

Subfamily RAILLIETIINAE Vitzthum, 1941

Genus **Raillietia** Trouessart, 1902

Type: *R. auris* (Leidy, 1872)	Ox
R. hopkinsi Radford, 1938	*Kobus defassa ugandae* Neumann, 1905

Subfamily RHINONYSSINAE Trouessart, 1895

Genus **Sternostomum** Trouessart, 1895

S. caledonicum (Hirst, 1921)	*Uria grylle* (L. 1758)
S. levenseni (Tragardh, 1904)	*Somateria mollisima* (L. 1758)
Type: *S. rhinolethrum* Trouessart, 1895	*Anser anser* L. 1758
S. technaui Vitzthum, 1935	*Cinclus cinclus aquaticus* Bechstein, 1803
S. waterstoni (Hirst, 1921)	*Alca torda* L. 1758

Genus **Sternostoma** Berlese & Trouessart, 1889

Type: *S. cryptorhynchum* Berlese & Trouessart, 1889	*Passer domesticus* (L. 1758)
S. tracheacolum Lawrence, 1948	Canary

Genus **Rhinonyssus** Trouessart, 1894

Type: *R. coniventris* Trouessart, 1894	*Arenaria interpres* (L. 1758)
R. echinipes Hirst, 1921	*Charadrius hiaticula* (L. 1758)
R. neglectus Hirst, 1921	*Calidris maritima* (Brunnich, 1764)

R. nitzschi (Giebel, 1871) — *Caprimulgus europaeus* L. 1758
R. novae-guineae Hirst, 1921 — *Craspidophora magnifica* (Viell, 1819)
R. orientalis Ewing, 1933 — *Lanius nigriceps longicaudatus* Gould, 1859
R. squamosus Vitzthum, 1935 — *Sericotes holosericeus*

Genus **Rhinonyssoides** Hirst, 1921

Type: *R. trouessarti* Hirst, 1921 — *Sphecotheres maxillaris* (Lath. 1790)

Genus **Rhinoecius** Cooreman, 1946

Type: *R. oti* Cooreman, 1946 — *Asio otus otus* (L. 1758)

Genus **Neonyssus** Hirst, 1921

Syn. *Neonyssoides* Hirst, 1923

Type: *N. intermedius* Hirst, 1921
N. nucifragae (Hirst, 1923) — *Nucifraga caryocatactes* (L. 1758)

Genus **Ptilonyssus** Berlese & Trouessart, 1889

Type: *P. echinatus* Berlese & Trouessart, 1889 — *Hirundo rustica* L. 1758
P. nudus Berlese & Trouessart, 1889 — *Passer domesticus* (L. 1758)
P. stresemanni Vitzthum, 1935 — *Procnias alba* (Hermann, 1783)

Genus **Ptilonyssoides** Vitzthum, 1935

Type: *P. triscutatus* Vitzthum, 1935 — *Merops apiaster* L. 1758

Genus **Larinyssus** Strandtmann, 1948

Type: *L. orbicularis* Strandtmann, 1948 — *Sterna maxima* Boddaert

Genus **Rallinyssus** Strandtmann, 1948

Type: *R. caudistigmus* Strandtmann, 1948 — *Fulica americana* Gmelin

Family IXODORHYNCHIDAE Fonseca, 1934

Genus **Ixodorhynchus** Ewing, 1922

Type: *I. liponyssoides* Ewing, 1922 — Snake

Genus **Ixobioides** Fonseca, 1934

Type: *I. butantanensis* Fonseca, 1934 — *Ophis merremii* Wagler, 1824

Family SPELAEORHYNCHIDAE Oudemans, 1902

Genus **Spelaeorhynchus** Neumann, 1902

S. latus Banks, 1917 — Bats
Type: *S. praecursor* Neumann, 1902 — Bats

Family SPINTURNICIDAE Oudemans, 1901

Genus **Spinturnix** von Heyden, 1826

S. abyssinicus Hirst, 1927 — Bat
S. acuminatus (Koch, 1836) — *Nyctalus noctula* Schreber, 1774
S. amboinensis Oudemans, 1925 — Bat
S. antipodianus Hirst, 1931 — Bat
S. araguensis Vitzthum, 1931 — *Myotis nigricans* Wiedemann, 1826
S. carloshoffmanni Hoffmann, 1944 — *Natalus mexicanus* Miller, 1902
S. euryalis (Canestrini, 1885) — *Rhinolophus euryale* Blasius, 1853
S. ewingia Wharton, 1938 — *Artibeus jamaicensis yucatanicus* (Allen, 1904)
S. iowae Keegan, 1938 — *Myotis lucifugus lucifugus* (Le Conte, 1831)
S. javensis Oudemans, 1914 — Bat
S. murinus Walkenaer, 1847 — *Myotis myotis* Borkhausen, 1797
S. novae-hollandiae Hirst, 1931 — Bat
S. omahonyi Turk, 1946 — *Rhinolophus hipposideros minutus*
S. oudemansi Eyndhoven, 1944 — *R. ferrum equinum* Schreber, 1774

S. plecotinus (Koch, 1836)	*Plecotus auritus* L. 1758
S. psi Kolenati, 1857	*Miniopteris schreibersi* Kuhl, 1817
S. semilunaris Meillon & Lavoipierre, 1944	*M. natalensis*
Type: *S. vespertilionis* (L. 1758)	*Myotis myotis* Borkhausen, 1797
S. vespertilionis ianzai Lombardini, 1944	*Rhinolophus* sp.

Genus **Ancystropus** Kolenati, 1856

A. aethiopicus Hirst, 1923	*Epomophorus minor* Dobson, 1880
A. jordani Radford, 1947	Bat
A. kenyaensis Radford, 1947	*Eidolon helvum* Kerr, 1792
Type: *A. zelebori* Kolenati, 1856	*Rhinopoma microphyllum* Brunnich, 1782
A. (*Meristaspis*) *calcaratus* Hirst, 1923	*Pteropus* sp.
A. (*Meristaspis*) *lateralis* Kolenati, 1856	*P. aegyptiaca* Geoffroy, 1810
A. (*Meristaspis*) *macroglossi* Hirst, 1923	*Macroglossus minimus* Geoffroy, 1810

Genus **Periglischrus** Kolenati, 1858

P. asema Kolenati, 1856	
P. caligus Kolenati, 1856	
P. glutinimargo Kolenati, 1856	*Rhinolophus clivosus* Cretzschmar, 1826
P. hipposideros Kolenati, 1856	*Rhinolophus hippocrepis*
Type: *P. interruptus* (Kolenati, 1856)	*R. blasii* Peters, 1866
P. jheringi Oudemans, 1902	*Vampyrops lineatus* Geoffroy, 1818
P. meridensis Hirst, 1927	*Artibeus jamaicensis* Leach, 1822

Genus **Tympanospinctus** Berlese, 1918

Type: *T. paradoxus* Berlese, 1918	*Crossarchus fasciatus*

Family PTERYGOSOMIDAE Oudemans, 1910

Genus **Pterygosoma** Peters, 1849

P. aculeatum Lawrence, 1936	*Agama hispida aculeata* Merrem, 1820
Type: *P. agamae* Peters, 1849	*A. mossambica mossambica* Peters, 1854
P. armatum Lawrence, 1936	*A. hispida armata* Peters, 1854
P. bedfordi Lawrence, 1936	*A. atra* Daudin, 1802
P. bicolor Lawrence, 1935	*Gerrhosaurus flavigularis flavigularis* Wiegmann, 1828
P. gerrhosauri Lawrence, 1935	*G. validus validus* Smith, 1849
P. hirsti Lawrence, 1936	*Agama atra* Daudin, 1802
P. hystrix Lawrence, 1935	*Gerrhosaurus flavigularis flavigularis* Wiegmann, 1828
P. inermis Tragardh, 1905	*Agama stellio stellio* (L. 1758)
P. longipalpe Lawrence, 1936	*A. atra* Daudin, 1802
P. melanum Hirst, 1917	*A. atra* Daudin, 1802
P. neumanni Berlese, 1910	*A. agama agama* (L. 1758)
P. persicum Hirst, 1917	*A. nupta* de Filippi, 1843
P. transvaalensis Lawrence, 1936	*A. atricollis* Smith, 1849
P. triangulare Lawrence, 1936	*A. hispida brachyura* Boulanger, 1885

Genus **Geckobia** Megnin, 1878

G. australis Hirst, 1926	*Lygodactylus capensis capensis* (Smith, 1849)
G. bataviensis Vitzthum, 1928	*Hemidactylus frenatus* Dum. & Bibr. 1836
G. boulengeri Hirst, 1917	*Hemiphyllodactylus yunnanensis* (Boulanger, 1903)
G. clelandi Hirst, 1917	*Platyurus platurus* (Schneid. 1792)
G. diversipilis Hirst, 1926	*Hemidactylus leschenaulti* Dum. & Bibr. 1836
G. gehyrae Hirst, 1926	*Peropus oceanica* (Lesson, 1830)
G. gleadoviana Hirst, 1926	*Hemidactylus brooki* Gray, 1845
G. gymnodactyli Womersley, 1941	*Gymnodactylus morio*
G. haplodactyli Womersley, 1941	*Hoplodactylus duvaucelli* (Dum. & Bibr. 1836)
G. hemidactyli Lawrence, 1936	*Hemidactylus tasmani* Hewitt, 1932
G. hewitti Lawrence, 1936	*Pachydactylus maculatus* Gray, 1845

G. hindustanica Hirst, 1926	*Hemidactylus leschenaulti* Dum. & Bibr. 1836
G. homopholis Lawrence, 1936	*Homopholis wahlbergii* (Smith, 1849)
G. indica Hirst, 1917	*Hemidactylus brooki* Gray, 1845
G. karrooica Lawrence, 1936	*Afroedura karrooica wilmoti* (Hewitt, 1926)
Type: *G. latasti* Megnin, 1878	*Platydactylus* sp.
G. loricata Berlese, 1892	*Tarentola mauritanica mauritanica* (L. 1758)
G. malayana Hirst, 1917	*Gymnodactylus pulchellus* (Gray, 1828)
G. manaensis Floch & Abonnenc, 1945	*Platydactylus* sp.
G. namaquensis Lawrence, 1935	*Pachydactylus namaquensis* (Sclater, 1899)
G. natalensis Lawrence, 1935	*Lygodactylus capensis capensis* (Smith, 1849)
G. naultina Womersley, 1941	*Naultinus* sp.
G. neumanni Berlese, 1910	*Agama agama agama* (L. 1758)
G. oedurae Lawrence, 1936	*Afroedura transvaalica platyceps* (Hewitt, 1925)
G. ovambica Lawrence, 1936	*Rhotropus barnardi* Hewitt, 1926
G. papuana Hirst, 1917	*Gymnodactylus louisiadensis* De Vis, 1892
G. phyllodactyli Lawrence, 1936	*Phyllodactylus porphyreus porphyreus* (Daudin, 1802)
G. platydactyli Lawrence, 1936	*Pachydactylus bibronii* Smith, 1846
G. rhoptropi Lawrence, 1936	*Rhoptropella ocellata* (Boulanger, 1885)
G. similis Tragardh, 1905	*Hemidactylus turcicus turcicus* L. 1758
G. simplex Hirst, 1926	*H. leschenaulti* Dum. & Bibr. 1836
G. socotrensis Hirst, 1917	*Pristurus rupestris* Blandford, 1874
G. tarantulae Tragardh, 1905	*Tarentula annulata*
G. tasmani Lawrence, 1936	*Hemidactylus tasmani* Hewitt, 1932
G. transvaalensis Lawrence, 1936	*Lygodactylus capensis capensis* (Smith, 1849)
G. turkestana Hirst, 1926	*Gymnodactylus russovi* Stranch, 1887

Genus **Hirstiella** Berlese, 1921

Type: *H. trombidiiformis* Berlese, 1921

Genus **Geckobiella** Hirst, 1917

Type: *G. texana* (Banks, 1905)	*Sceloporus floridanus* (Baird, 1858)

Genus **Pimeliaphiloides** Vitzthum, 1941

Type: *P. insignis* (Berlese, 1892)	*Tarentola mauritanica mauritanica* (L. 1758)

Genus **Ixodiderma** Lawrence, 1935

Type: *I. inverta* Lawrence, 1935	*Pseudocordylus subviridis* Smith, 1838
I. lacertae Lawrence, 1935	*Scaptira suborbitalis* (Peters, 1869)
I. pilosa Lawrence, 1935	*Pseudocordylus subviridis* Smith, 1838

Genus **Scaphothrix** Lawrence, 1935

Type: *S. convexa* Lawrence, 1935	*Cordylus polyzonus* Smith, 1838

Genus **Zonurobia** Lawrence, 1935

Z. circularis capensis Lawrence, 1935	*Platysaurus capensis* Smith, 1844
Z. circularis circularis Lawrence, 1935	*P. guttatus* Smith, 1849
Z. circularis latior Lawrence, 1935	*Cordylus vittifer* (Reichenow, 1887)
Z. circularis longipilis Lawrence, 1935	*Platysaurus guttatus* Smith, 1849
Z. circularis spiniventer Lawrence, 1935	*P. minor minor* Fitz Simons, 1930
Z. circularis transvaalensis Lawrence, 1935	*Platysaurus wilhelmi* Hewitt, 1909
Type: *Z. cordylensis* Lawrence, 1935	*Cordylus cordylus cordylus* (L. 1758)
Z. debilipes Lawrence, 1935	*C. warreni warreni* (Boulanger, 1908)
Z. montana Lawrence, 1935	*C. namaquensis* (Meth. & Hewitt, 1914)
Z. polyzonensis Lawrence, 1935	*C. polyzonus* Smith, 1838
Z. sanguinea Lawrence, 1935	*C. coeruleopunctatus* (Meth. & Hewitt, 1913)
Z. semilunaris Lawrence, 1935	*Pseudocordylus microlepidotus microlepidotus* (Cuvier, 1829)
Z. subquadrata Lawrence, 1935	*Cordylus capensis robertsi* (van Dam, 1921)
Z. transvaalica Lawrence, 1935	*C. vandami vandami* (Fitz Simons, 1930)

Family CHEYLETIDAE Leach, 1814

Genus **Syringophilus** Haller, 1880

Type: *S. bipectinatus* Haller, 1880	*Gallus domesticus* L. 1758
S. columbae Hirst, 1920	*Columba domestica*
S. totani Oudemans, 1904	*Totanus totanus* L.

Genus **Picobia** Haller, 1878

Type: *P. heeri* Haller, 1878	*Gecinus canus*

Genus **Harpyrynchus** Megnin, 1878

H. crista-galli Berlese & Trouessart, 1889	*Colius striatus* (Gmelin, 1789)
H. holopus Berlese & Trouessart, 1889	*Passer domesticus* (L. 1758)
H. longipilis Banks, 1904	*Loxia* sp.
Type: *H. nidulans* (Nitzsch, 1818)	*Alauda arvensis* L. 1758
H. pilirostris Berlese & Trouessart, 1889	*Passer domesticus* (L. 1758)
H. tabescentium (Berthold, 1845)	Man

Genus **Psorergates** Tyrrell, 1883

P. ovis Womersley, 1941	Sheep
Type: *P. simplex* Tyrrell, 1883	Mouse

Genus **Cheyletiella** Canestrini, 1885

C. canadensis Banks, 1909	Bluebird
C. chanay Berlese & Trouessart, 1892	*Fringilla coelebs* L. 1758
C. curvidens Lawrence, 1948	*Myonax cauui punctulatus* (Gray, 1849)
C. heteropalpus (Megnin, 1878)	*Cypselus obvia*
C. lemuricola Lawrence, 1948	*Galago crassicaudatus garnetti* (Ogilby, 1838)
C. macronychus (Megnin, 1878)	Passerines
Type: *C. parasitivorax* (Megnin, 1878)	Rabbit

Syn. *Ewingella americana* Vail & Augustson, 1943

C. pinguis Berlese, 1889	*Merula nigra*

Genus **Ophioptes** Sambon, 1928

O. coluber Radford, 1946	*Elaphe radiata* (Schlegel, 1851)
O. oudemansi Sambon, 1928	*Pseudoboa rustica* Cope, 1877
Type: *O. parkeri* Sambon, 1928	*Erythrolamprus aesculapii* (L. 1758)
O. tropicalis Ewing, 1933	*Erpetodryas carinatus* (L. 1754)

Family MYOBIIDAE Megnin, 1877

Subfamily Myobiinae Ewing, 1938

Genus **Myobia** von Heyden, 1826

M. etheldredae (Perkins, 1925)	*Pipistrellus pipistrellus* Schreber, 1774
M. longa Ewing, 1938	*Tadarida mexicana* Saussure, 1860
Type: *M. muris-musculi* (Schrank, 1781)	*Mus musculus musculus* L. 1758

Syn. *Myobia coarctata* von Heyden, 1826

M. simplex Ewing, 1938	*Blarina brevicauda talpoides* Gapper, 1830
M. stewardi Radford, 1948	*Rattus rattus rufescens* Gray, 1837

Genus **Amorphacarus** Ewing, 1938

Type: *A. elongata* (Poppe, 1896)	*Sorex araneus* L. 1758
A. hengererorum Jameson, 1948	*S. fumeus* Miller, 1895

Genus **Protomyobia** Ewing, 1938

P. brevisetosa Jameson, 1948	*Sorex fumeus* Miller, 1895
Type: *P. claparedei* (Poppe, 1896)	*S. araneus* L. 1758

Genus **Radfordia** Ewing, 1938

R. aegyptica Radford, 1948	*Gerbillus* sp.
R. affinis (Poppe, 1896)	*Mus musculus musculus* L. 1758
R. blairi (Radford, 1936)	*Crocidura cassiteridium* Hinton, 1924
R. capensis (Meillon & Lavoipierre, 1944)	*Eptesicus capensis gracilior* Thomas & Schwann, 1905
R. caudata (Banks, 1909)	Little brown bat
	Syn. *Myobia canadensis* Banks, 1909
R. ceylonica Radford, 1948	*Hipposideros brachyotis* Dobson, 1874
R. chalinolobus (Womersley, 1941)	*Chalinolobus gouldi* Gray, 1841
R. clara (Womersley, 1941)	Bat
R. davisi (Radford, 1938)	*Rattus norvegicus norvegicus* Berkenhout, 1769
Type: *R. ensifera* (Poppe, 1896)	*R. norvegicus norvegicus* Berkenhout, 1769
	Syn. *Myobia ratti* Skidmore, 1934
R. ewingi (Fox, 1937)	*Zapus hudsonicus americanus* Barton, 1799
R. floridensis Ewing, 1938	*Tadarida cynocephala* Le Conte, 1831
R. heteronycha (Berlese & Trouessart, 1889)	*Asellia tridens* Geoffroy, 1813
R. inaequalis Ewing, 1938	*Tadarida cynocephala* Le Conte, 1831
R. ingens (Vitzthum, 1914)	*Pachyura etrusca* Savi, 1822
R. lancearia (Poppe, 1908)	*Apodemus sylvaticus sylvaticus* L. 1758
R. lemnina (Koch, 1835)	*Microtus agrestis hirtus* Bellamy, 1839
R. magna (Radford, 1934)	*Pteropus giganteus* Brunnich, 1782
R. michaeli (Poppe, 1896)	*Crocidura russula* Hermann, 1780
R. minuta (Radford, 1940)	Bat
R. multivaga (Poppe, 1908)	*Micromys minutus soricinus* Pallas, 1769
R. noctulia (Radford, 1940)	*Nyctalus noctula* Schreber, 1774
R. oudemansi (Poppe, 1908)	*Muscardinus avellanarius avellanarius* L. 1758
R. phillipsi (Turk, 1945)	*Neomys fodiens bicolor* Shaw, 1791
R. sigmodontis Radford, 1948	*Sigmodon hispidus texianus* Audubon & Bachman, 1853
R. subuliger Ewing, 1938	*Reithrodontomys humulis impiger* Bangs, 1898
R. trouessarti (Poppe, 1896)	*Potamogale velox* Du Chaillu, 1860
R. zibethicalis (Radford, 1936)	*Ondatra zibethica zibethica* L. 1766

Genus **Eadiea** Jameson, 1949

E. brevihamata (Haller, 1882)	*Mustela nivalis* L. 1766
Type: *E. condylurae* Jameson, 1949	*Condylura cristata* (L. 1758)

Genus **Eutalpacarus** Jameson, 1949

Type: *E. peltatus* Jameson, 1949	*Neurotrichus gibbsii* (Baird)

Genus **Calcarmyobia** Radford, 1948

Type: *C. rhinolophia* (Radford, 1940)	*Rhinolophus lobatus* Peters, 1867
	Syn. *Myobia miniopteris* Womersley, 1941

Genus **Neomyobia** Radford, 1948

N. fortuitous Radford, 1948	*Scotophilus wroughtoni* Thomas, 1897
N. jacksoni (Radford, 1940)	Bat
N. pantopus (Poppe & Trouessart, 1895)	*Barbastella barbastellus* Schreber, 1774
N. pipistrellia (Radford, 1938)	*Pipistrellus pipistrellus* Schreber, 1774
N. plecotia (Radford, 1938)	*Plecotus auritus* L. 1758
N. poppei (Trouessart, 1895)	*P. nathusii* Keys & Blasius, 1839
Type: *N. rollinati* (Poppe, 1908)	*Rhinolophus ferrum equinum* Schreber, 1774

Genus **Ewingana** Radford, 1948

Type: *E. bispinosa* Radford, 1948	*Nyctinomus cisturus* Thomas, 1903

Genus **Foliomyobia** Radford, 1948

Type: *F. chiropteralis* (Michael, 1884)	*Rhinolophus hipposideros* Bechstein, 1789
F. mystacinalis (Radford, 1935)	*Myotis mystacinus* Kuhl, 1819

Family DEMODICIDAE Nicolet, 1855

Genus **Demodex** Owen, 1843

D. arvicolae Zschokke, 1888	*Microtus agrestis agrestis* L. 1761
D. arvicolae var. *apodemi* Hirst, 1918	*Apodemus sylvaticus sylvaticus* L. 1758
D. arvicolae var. *glareoli* Hirst, 1919	*Evotomys glareolus britannicus* Miller, 1900
D. arvicolae var. *musculi* Oudemans, 1898	*Mus musculus musculus* L. 1758
D. bovis Stiles, 1892	*Bos taurus* L. 1758
D. canis Leydig, 1859	*Canis familiaris* L. 1766
D. canis var. *erinacei* Hirst, 1917	*Erinaceus europaeus* L. 1758
D. canis var. *ovis* Railliet, 1895	*Ovis aries* L. 1758
D. caprae Railliet, 1895	*Capra hircus* L. 1758
D. cati Megnin, 1877	*Felis domesticus*
D. cervi Preitsch, 1886	*Cervus* sp.
D. chiropteralis Hirst, 1921	*Plecotus auritus* L. 1758
D. cuniculi Pfeiffer, 1903	*Oryctolagus cuniculus* (L. 1758)
D. equi Railliet, 1895	*Equus caballus* L. 1758
D. ermineae Hirst, 1919	*Mustela erminea* L. 1758
Type: *D. folliculorum* (Simon, 1843)	Man
D. gliricolens Hirst, 1921	*Arvicola amphibius amphibius* L. 1758
D. longior Hirst, 1918	*Apodemus sylvaticus sylvaticus* L. 1758
D. melesinus Hirst, 1921	*Taxidea taxus* (Schreber)
D. muscardini Hirst, 1917	*Muscardinus avellanarius avellanarius* L. 1758
D. nanus Hirst, 1918	*Rattus rattus rattus* L. 1758
D. phylloides Csokor, 1879	*Sus scrofa* L.
D. phyllostomatis Leydig, 1859	*Phyllostoma hastatum* (Pallas, 1766)
D. ratti Hahn	*Rattus norvegicus norvegicus* Berkenhout, 1769
S. sciurinus Hirst, 1923	*Sciurus vulgaris vulgaris* L. 1758
D. soricinus Hirst, 1918	*Rattus rattus rattus* L. 1758
D. talpae Hirst, 1921	*Talpa europaea* L. 1758

Family SARCOPTIDAE Trouessart, 1892

Genus **Sarcoptes** Latreille, 1806

S. aucheniae Railliet, 1893	*Lama glama* (L. 1758)
S. canis Gerlach, 1857	*Canis familiaris* L. 1766
S. caprae Furstenberg, 1861	*Capra hircus* L. 1758
S. cuniculi Neumann, 1892	*Oryctolagus cuniculus* (L. 1758)
S. dromedarii Gervais, 1841	*Camelus dromedarius* L. 1758
S. equi Gerlach, 1857	*Equus caballus* L. 1758
S. furonis Railliet, 1893	*Mustela furo* L. 1758
S. hydrochoeri Megnin, 1880	*Hydrochoerus hydrochoerus* (L. 1758)
S. leonis Canestrini, 1862	*Felis leo* L. 1758
S. lupi Megnin, 1880	*Canis lupus* L. 1758
S. ovis Megnin, 1880	*Ovis aries* L. 1758
S. parvulus Canestrini, 1894	*Sus domesticus*
S. rupricaprae Hering, 1838	*Rupicapra rupicapra* (L. 1758)
Type: *S. scabiei* (Degeer, 1778)	Man
S. scabiei-crustosae Furstenberg, 1861	Man
S. suis Gerlach, 1857	*Sus domesticus*
S. vulpis Furstenberg, 1861	*Canis vulpes* L. 1758
S. wombati Railliet, 1893	*Phascolomys ursinus* (Shaw)

Genus **Notoedres** Railliet, 1893

Type: *N. cati* (Hering, 1838)	*Felis domesticus*
N. cuniculi (Gerlach, 1857)	*Oryctolagus cuniculus* (L. 1758)
N. muris Megnin, 1880	*Rattus rattus rattus* L. 1758

Genus **Prosopodectes** Canestrini, 1897

Type: *P. chiropteralis* (Trouessart, 1896) *Rhinolophus ferrum equinum* Schreber, 1774

Genus **Nycteridocoptes** Oudemans, 1897

Type: *N. poppei* Oudemans, 1897 *Myotis murinus* L. 1758

Genus **Teinocoptes** Rodhain, 1923

Type: *T. epomorphi* Rodhain, 1923 Bat

Genus **Cnemidocoptes** Furstenberg, 1870

Syn. *Knemidokoptes* Furstenberg, 1870

C. fossor (Ehlers, 1873) *Munia major*
C. gallinae (Railliet, 1887) *Gallus domesticus* L. 1758
C. glabberimus Sicher, 1893 *Dendrocopus medius* (L. 1758)
C. laevis (Railliet, 1887) *Columba domestica* L. 1758
Type: *C. mutans* (Robin, 1860) *Gallus domesticus* L. 1758

Syn. *Knemidokoptes viviparus* Furstenberg, 1870

C. philomelae Sicher, 1893 *Erithacus luscinia* (L. 1758)

Family PSORALGIDAE Oudemans, 1908

Genus **Psoralges** Trouessart, 1896

Type: *P. libertus* Trouessart, 1896 *Tamandua tetradactyla* (L. 1758)

Family PSOROPTIDAE Canestrini, 1892

Genus **Psoroptes** Gervais, 1841

P. bovis (Gerlach, 1857) *Bos taurus* L. 1758
P. cuniculi (Delafond, 1859) *Oryctolagus cuniculus* (L. 1758)
Type: *P. equi* (Hering, 1838) *Equus caballus* L. 1758
P. gazellae Canestrini, 1894 *Gazella* sp.
P. natalensis Hirst, 1917 Buffalo
P. ovis (Hering, 1838) *Ovis aries* L. 1758

Genus **Chorioptes** Gervais, 1859

C. bovis (Gerlach, 1857) *Bos taurus* L. 1758
Type: *C. caprae* Gervais, 1859 *Capra hircus* L. 1758
C. cuniculi (Zurn, 1874) *Oryctolagus cuniculus* (L. 1758)
C. equi (Gerlach, 1857) *Equus caballus* L. 1758
C. ovis Railliet, 1893 *Ovis aries* L. 1758

Genus **Otodectes** Canestrini, 1894

Type: *O. cynotis* (Hering, 1838) *Canis familiaris* L. 1758
O. furonis (Railliet, 1893) *Mustela furo*

Genus **Caparinia** Canestrini, 1894

Type: *C. setifera* (Megnin, 1880) *Hyaena* sp.
C. tripilis (Michael, 1889) *Erinaceus europaeus* L. 1758
C. vulpis (Megnin, 1880) *Canis vulpes* L. 1758

Family LAMINOSIOPTIDAE Vitzthum, 1931

Genus **Laminosioptes** Megnin, 1879

Type: *L. cysticola* (Vizioli, 1870) Phasianidae

Family CYTOLEICHIDAE Canestrini, 1892

Genus **Cytoleichus** Megnin, 1879

C. banksi Wellman & Wherry, 1910 *Citellus beecheyi beecheyi* Richardson, 1829
C. hominis Hirst, 1917 Man
Type: *C. nudus* (Vizioli, 1870) *Phasianus colchicus* L. 1758
C. penrosei Weidman, 1917 *Cynomys ludovicianus* Ord, 1815

Family LISTROPHORIDAE Canestrini, 1892

Genus **Listrophorus** Pagenstecher, 1861

L. americanus Radford, 1944	*Ondatra zibethica macrodon* Merriam, 1897
L. argentinus Hirst, 1921	*Scapteromys tomentosus* Lichtenstein, 1829
L. bakeri Radford, 1948	*Sigmodon hispidus texianus* Audubon & Bachman, 1933
L. bothae Hirst, 1923	Gerbille
L. brevicaudatus Ullrich, 1939	*Lepus timidus* L. 1758
L. cucullatus Trouessart, 1892	*Rattus norvegicus norvegicus* Berkenhout, 1769
L. dipodicola Tragardh, 1904	*Gerbillus gerbillus gerbillus* Olivier, 1801
L. dozieri Radford, 1944	*Ondatra zibethica macrodon* Merriam, 1897
L. floridanus Radford, 1948	*Geomys tuza tuza* Barton, 1806
L. frontalis Hirst, 1921	*Oryzomys delticola* Thomas, 1917
L. genettus Radford, 1944	Genet
L. gibbus Pagenstecher, 1861	*Oryctolagus cuniculus* (L. 1758)
Type: *L. leuckarti* Pagenstecher, 1861	*Microtus arvalis arvalis* Pallas, 1778
L. lophuromys Radford, 1940	*Lophuromys sikapusi sikapusi* Temminck, 1853
L. mustelae Megnin, 1885	*Mustela nivalis* L. 1766
L. pagenstecheri Haller, 1880	*Sciurus vulgaris vulgaris* L. 1758
L. suncus Radford, 1947	*Suncus caeruleus giganteus* Geoffroy, '837
L. validus Banks, 1909	Musk rat

Genus **Eurychiroides** Womersley, 1943

Syn. *Euryzonus* Trouessart, 1917

Type: *E. ventricosus* (Trouessart, 1917)	*Sigmodon* sp.

Subfamily MYOCOPTINAE Gunther, 1942

Genus **Myocoptes** Claparede, 1869

M. criceti Poppe, 1887	*Cricetus cricetus cricetus* L. 1758
M. glirinus Canestrini, 1895	*Glis glis glis* L. 1766
M. hintoni Hirst, 1919	*Sciurus vulgaris vulgaris* L. 1758
M. kalrai Radford, 1947	*Gerbillus* sp.
Type: *M. musculinus* (Koch, 1844)	*Mus musculus musculus* L. 1758

Syn. *Listrophorus larisi* Vorobiov, 1938

M. ramboutsi Eyndhoven, 1946	*Mus musculus brunnea*
M. stigmatus Fox, 1946	*Rattus rattus rattus* L. 1758
M. tenax Michael, 1889	*Microtus arvalis arvalis* Pallas, 1778

Genus **Trichoecius** Canestrini, 1899

Type: *T. brevipes* (Canestrini & Trouessart, 1895)	*Arvicola arvalis arvalis* Pallas, 1778

Subfamily ATOPOMELINAE Gunther, 1942

Genus **Atopomelus** Trouessart, 1917

Type: *A. locusta* Trouessart, 1917	*Neotetracus sinensis* Trouessart, 1909

Genus **Marquesania** Womersley, 1943

Type: *M. expansus* (Ferris, 1932)	*Rattus* sp.
M. expansus queenslandica Womersley, 1943	*Rattus culmorum youngi* Thomas, 1926

Genus **Campylochirus** Trouessart, 1893

Syn. *Chirodiscoides* Hirst, 1917

C. adherens Trouessart, 1893	*Anomalurus fraseri erythronotus* Milne-Edwards, 1879
C. caviae (Hirst, 1917)	*Cavia*
Type: *C. chelopus* Trouessart, 1893	*Pseudocheirus convoluta* Oken, 1816
C. latus Trouessart	*Capromys pilorides pilorides* Say, 1822

Genus **Austrochirus** Womersley, 1943

Type: *A. queenslandicus* Womersley, 1943	Possum

Genus **Listrophoroides** Hirst, 1923

Type: *L. aethiopicus* Hirst, 1923	*Cricetomys gambianus gambianus* Waterhouse, 1840
L. africana Radford, 1944	*Mastomys coucha erythroleucus* Temminck, 1853
L. bathyergians Radford, 1939	*Bathyergus suillus suillus* Schreber, 1782
L. dasymys Radford, 1942	*Dasymys* sp.
L. leggadilla Radford, 1947	*Leggadilla fernandoni* Phillips, 1932
L. lemniscomys Radford, 1940	*Lemniscomys striatus massaicus* Pagenstecher, 1884
L. mastomys Radford, 1940	*Mastomys coucha erythroleucus* Temminck, 1853
L. tragardhi Radford, 1940	*Rattus norvegicus norvegicus* Berkenhout, 1769

Subfamily LABIDOCARPINAE Gunther, 1942

Genus **Labidocarpus** Trouessart, 1895

Type: *L. rollinati* Trouessart, 1895	*Rhinolophus ferrum equinum* Schreber, 1774

Genus **Alabidocarpus** Ewing, 1929

Type: *A. megalonyx* (Trouessart, 1895)	*Rhinolophus ferrum equinum* Schreber, 1774
A. minor (Trouessart, 1897)	*R. ferrum equinum* Schreber, 1774
A. nasicolus (Lawrence, 1938)	*R. augur zuluensis* Andersen, 1904
A. recurvus (Womersley, 1943)	Bat

Genus **Olabidocarpus** Lawrence, 1948

Type: *O. belsorum* (Eyndhoven, 1940)	*Myotis myotis* Borkhausen, 1797

Genus **Eulabidocarpus** Lawrence, 1948

Type: *E. compressus* (Ewing, 1910)	*Pteropus giganteus* Brunnich, 1782

Genus **Neolabidocarpus** Gunther, 1942

Type: *N. buloloensis* (Gunther, 1940)	*Macropus* (*Thylogale*) *coxeni* Gray

Genus **Prolabidocarpus** Lawrence, 1948

Type: *P. canadensis* Lawrence, 1948	*Castor canadensis* L. 1758

Genus **Chirodiscus** Trouessart & Neumann, 1890

Type: *C. amplexans* Trouessart & Neumann, 1890	*Podargus strigoides*

Genus **Schizocarpus** Trouessart, 1896

Type: *S. mingaudi* Trouessart, 1896	*Castor fiber fiber* L. 1758

Genus **Schizocoptes** Lawrence, 1944

Type: *S. conjugatus* Lawrence, 1944	*Bematiscus villosus dobsoni* Broom, 1918

HOST LIST

Cercopithecus schmidti Matschie, 1892	Putty-nosed monkey
Macaca mulatta Zimmerman, 1780	Rhesus monkey
M. nemestrina L. 1766	Pig-tailed macaque
Papio porcarius Boddaert, 1787	Chacma baboon
Lagothrix infumatus Spix, 1823	Brown woolley monkey
Lemur mongoz albifrons Geoffroy, 1796	Lemur
Galago crassicaudatus garnetti (Ogilby, 1838)	South African galago
Pteropus edulis Geoffroy, 1810	Fruit bat
P. giganteus Brunnich, 1782	Indian fruit bat
Eidolon helvum Kerr, 1792	Straw-coloured fruit bat
Rousettus aegyptiaca Geoffroy, 1810	Egyptian fruit bat
Macroglossus minimus Geoffroy, 1810	Long-tongued fruit bat
Rhinolophus augur zuluensis Andersen, 1904	Horse-shoe bat
R. blasii Peters, 1866	Horse-shoe bat
R. clivosus Cretzschmar, 1826	Arabian horse-shoe bat

Rhinolophus euryale Blasius, 1857	Horse-shoe bat
R. ferrum-equinum Schreber, 1774	Greater horse-shoe bat
R. hippocrepis	Horse-shoe bat
R. hipposideros hipposideros Bechstein, 1789	Lesser horse-shoe bat
R. hipposideros minutus	Lesser horse-shoe bat
R. lobatus Peters, 1867	Peter's horse-shoe bat
Hipposideros brachyotis Dobson, 1874	Dekkhan leaf-nosed bat
Asellia tridens Geoffroy, 1813	Trident bat
Phyllostoma hastatum (Pallas, 1766)	Javelin bat
Glossophaga soricina (Pallas, 1766)	Shrew-like vampire
Artibeus jamaicensis jamaicensis Leach, 1822	False vampire
A. jamaicensis lituratus Lichtenstein	
Artibeus jamaicensis yucatanicus (Allen, 1940)	
Desmodus rotundus rotundus (Geoffroy, 1810)	Vampire bat
Natalus mexicanus Miller, 1902	Crowned bat
Epomophorus minor Dobson, 1880	Lesser epauletted bat
Rhinopoma microphyllum Brunnich, 1782	Long-tailed bat
Vampyrops lineatus Geoffroy, 1810	
Myotis dasycneme Boie, 1825	Mouse-eared bat
M. daubentonii Kuhl, 1819	Daubenton's bat
M. lucifugus lucifugus (Le Conte, 1831)	Little brown bat
M. murinus L. 1758	Mouse-coloured bat
M. myotis Borkhausen, 1797	Mouse-eared bat
M. mystacinus Kuhl, 1819	Whiskered bat
M. nattereri Kuhl, 1819	Natterer's bat
M. nigricans nigricans (Wiedemann,'1826)	South American mouse-eared bat
M. ruber (Geoffroy, 1806)	
M. subulatus subulatus (Say, 1823)	
Pipistrellus nathusii Keys & Blasius, 1839	Pipistrelle bat
P. pipistrellus Schreber, 1774	Pipistrelle bat
Nyctalus noctula Schreber, 1774	Noctule bat
Eptesicus capensis gracilior Thomas & Schwann, 1905	Natal serotine bat
E. serotinus Schreber, 1774	Serotine bat
Nycticeius humeralis Rafinesque, 1818	Rafinesque's evening bat
Lyponycteris nudiventris Cretzschmar, 1826	Tomb bat
Chalinolobus gouldi Gray, 1841	Gould's bat
Barbastella barbastellus Schreber, 1774	Barbastelle bat
Plecotus auritus L. 1758	Long-eared bat
Miniopteris schreibersi Kuhl, 1817	Long-fingered bat
Scotophilus wroughtoni Thomas, 1897	Wroughton's bat
Nyctinomus brasiliensis Geoffroy, 1824	Wrinkle lipped bat
N. cisturus Thomas, 1903	Wrinkle lipped bat
Molossus nasutus Spix, 1823	Wrinkle lipped bat
Tadarida brasiliensis Geoffroy, 1824	Free-tailed bat
T. cynocephala Le Conte, 1831	Free-tailed bat
T. mexicana Saussure, 1831	Free-tailed bat
Eumops abrassus abrassus (Temminck, 1827)	
Tupaia picta Thomas, 1892	Tree shrew
Macroscelides proboscideus (Shaw, 1800)	Elephant shrew
Erinaceus europaeus L. 1758	Hedgehog
Neotetracus sinensis Trouessart, 1909	Szechuen hedgehog shrew
Sorex araneus L. 1758	Common shrew
S. fumeus Miller, 1895	Smokey shrew
S. minutus L. 1766	Pygmy shrew
Blarina brevicauda talpoides Gapper, 1830	American short-tailed shrew
Neomys fodiens bicolor (Shaw, 1791)	Water shrew
Crocidura cassiteridium Hinton, 1924	White-toothed shrew
C. russula Hermann, 1780	Shrew

Suncus caeruleus giganteus Geoffroy, 1837	Musk shrew
Pachyura etrusca Savi, 1822	Musk shrew
Talpa europaea L. 1758	Common mole
Solenodon paradoxus Brdt. 1833	Haytian solenodon
Potamogale velox Du Chaillu, 1860	Ottershrew
Bematiscus villosus dobsoni Broom, 1918	South African golden mole
Scalopus aquaticus (L. 1758)	Mole
S. argentatus Audubon & Bachman, 1842	Mole
Felis domesticus	Cat
F. leo L. 1758	Lion
Canis familiaris L. 1766	Dog
C. lupus L. 1758	European wolf
Vulpes vulpes (L. 1758)	Fox
Mungos mungo macrurus Thomas	Banded mongoose
Myonax cauui punctulatus (Gray, 1849)	Slender mongoose
Mustela erminea L. 1758	Stoat
M. furo L. 1758	
M. nivalis L. 1766	Common weasel
Galictis vittata Schreber, 1775	Weasel
Mephitis nigra (Peale & Beauvois, 1796)	Skunk
Otaria byronia (de Blainville, 1820)	Southern sea lion
Odobenus rosmarus (L. 1758)	Atlantic walrus
Halichoerus grypus (Fabricius, 1791)	Grey seal
Mirounga angustirostris (Gill, 1866)	Northern elephant seal
Monachus tropicalis (Gray, 1850)	West Indian seal
Leptonychotes weddelli (Lesson, 1826)	Weddell's seal
Zalophus californianus (Lesson, 1866)	Californian sea lion
Bathyergus suillus suillus Schreber, 1782	Cape dune mole
Georychus capensis capensis Pallas, 1779	Cape blesmol
Cryptomys hottentotus hottentotus Lesson, 1826	Mole rat
Isothrix bistriatus bistriatus Wagner, 1845	
Euryzygomatomys spinosus catellus Thomas, 1916	Spiny rat
Cuniculus paca paca L. 1766	Agouti
Cavia aperea aperea Erxleben, 1777	Cavy
Galea spixii Wagler, 1831	Cavy
Caviella australis australis Geoffroy & D'Orbigny, 1833	Cavy
Sciurus aestuans aestuans L. 1766	South American squirrel
S. vulgaris vulgaris L. 1758	Red squirrel
Callosciurus quinquistriatus quinquistriatus Anderson, 1871	Five-striped squirrel
Tamiasciurus hudsonicus richardsoni Bachman, 1838	Richardson's red squirrel
Protoxerus stangeri centricola Thomas, 1906	Squirrel
Citellus beecheyi beecheyi Richardson, 1829	Ground squirrel
Cynomys ludovicianus ludovicianus Ord, 1815	Prairie dog
Castor canadensis L. 1758	Beaver
C. fiber fiber L. 1758	Beaver
Geomys bursarius bursarius Shaw, 1800	Pocket gopher
G. tuza tuza Barton, 1806	Gopher
Anomalurus fraseri erythronotus Milne-Edwards, 1879	Scaly-tailed squirrel
Zapus hudsonicus americanus Barton, 1799	Jumping mouse
Glis glis glis L. 1766	Dormouse
Muscardinus avellanarius avellanarius L. 1758	Dormouse
Spalax microphthalmus Guldenstaedt, 1770	Mole rat
S. monticola thermaicus Hinton, 1920	Mole rat
Micromys minutus soricinus Pallas, 1769	Harvest mouse
Apodemus agrarius agrarius Pallas, 1778	Striped field mouse
A. sylvaticus sylvaticus L. 1758	Wood mouse
Dasymys incomtus helukus Heller, 1910	East African swamp rat
Arvicanthis abyssinicus rubescens Wroughton, 1909	Uganda unstriped grass-rat

Lemniscomys griselda spinalis Thomas, 1916	Striped rat
L. striatus massaicus Pagenstecher, 1884	Masai striped grass-rat
Rhabdomys pumilio pumilio Sparrmann, 1784	Striped mouse
Hybomys trivirgatus trivirgatus Temminck, 1853	Three-striped bush mouse
Aethomys chrysophilus chrysophilus de Winton, 1896	African bush rat
Thallomys namaquensis grahami Roberts, 1915	Graham's rock mouse
Rattus hawaiiensis Stone, 1917	Hawaii rat
R. norvegicus norvegicus Berkenhout, 1769	Brown rat
R. rattus rattus L. 1758	Black rat
R. rattus rufescens Gray, 1837	
R. whiteheadi whiteheadi Thomas, 1894	Whitehead's rat
Dephomys defua Miller, 1900	Bristle-haired rat
Mastomys coucha erythroleucus Temminck, 1853	Multimammate rat
M. coucha hildebrandti Peters, 1878	Multimammate rat
Gyomys apodemoides Finlayson, 1932	Finlayson's mouse
Melomys cervinipes littoralis Lonneberg, 1916	Scale-tailed rat
M. rufescens rufescens Alston, 1877	Mosaic-tailed rat
Mus musculus algirus Pomel, 1856	Algerian mouse
M. musculus musculus L. 1758	House mouse
Leggadilla fernandoni Phillips, 1932	Ceylon spiny-mouse
Lophuromys aquilus aquilus True, 1892	Speckled, harsh-furred mouse
L. aquilus zena Dollman, 1909	Brush-furred mouse
L. sikapusi sikapusi Temminck, 1853	Brush-furred mouse
Acomys cahirinus Desmarest, 1819	Spiny mouse
Saccostomys campestris campestris Peters, 1846	Peter's pouched mouse
Cricetomys gambianus gambianus Waterhouse, 1840	Gambian pouched mouse
Oryzomys delticola Thomas, 1917	Uruguay mouse
O. eliurus Wagner, 1845	Mouse
O. subflavus Wagner, 1842	Vesper mouse
Reithrodontomys humulis impiger Bangs, 1898	American harvest mouse
Peromyscus californicus californicus Gambel, 1848	Parasitic mouse
P. eremicus eremicus Baird, 1857	Desert mouse
P. truei truei Shufeldt, 1886	White-footed mouse
Onychomys leucogaster arcticeps Rhoads, 1898	Grasshopper mouse
Akodon olivaceus Waterhouse, 1837	Mouse
Zygodontomys lasiurus Lund, 1841	
Scapteromys tomentosus Lichtenstein, 1829	Uruguay mouse
Phodopus roborovskii bedfordiae Thomas, 1908	Hamster
Cricetus cricetus cricetus L. 1758	Hamster
Mystromys albicaudatus albicaudatus Smith, 1834	White-tailed rat
Chinchillula sahamae Thomas, 1898	
Reithrodon cuniculoides cuniculoides Waterhouse, 1837	Rabbit rat
Holochilus sciureus Wagner, 1842	Water rat
H. vulpinus Lichtenstein, 1827	Water rat
Sigmodon hispidus texianus Audubon & Bachman, 1853	Cotton rat
Neotoma lepida lepida Thomas, 1893	Wood rat
Tachyoryctes ruddi ruddi Thomas, 1909	Mt Elgon mole rat
Gerbillus gerbillus gerbillus Olivier, 1801	Lesser Egyptian gerbille
Tatera benvenuta benvenuta Hinton & Kershaw, 1920	Gerbille
T. brantsi brantsi Smith, 1836	Gerbille
Myospalax cansus cansus Lyon, 1907	Mole rat
Lemmus obensis obensis Brants, 1827	Siberian lemming
Clethrionomys dawsoni dawsoni Merriam, 1888	Red-backed mouse
C. glareolus britannicus Miller, 1900	Bank vole
Phenacomys albipes Merriam, 1901	Coast mouse
Microtus agrestis hirtus Bellamy, 1839	Field vole
M. arvalis arvalis Pallas, 1778	Small vole
M. californicus californicus Peale, 1848	Californian vole

Microtus orcadensis westrae Miller, 1908	Orkney vole
Arvicola amphibius amphibius L. 1758	Water rat
Ondatra zibethica macrodon Merriam, 1897	Black musk-rat
O. zibethica zibethica L. 1766	Musk-rat
Oryctolagus cuniculus (L. 1758)	Common rabbit
Lepus timidus L. 1758	Common hare
Bos taurus L. 1758	Cattle
Ovis aries L. 1758	Sheep
Capra hircus L. 1758	Goat
Camelus dromedarius L. 1758	Dromedary
Lama glama (L. 1758)	Llama
Sus domesticus	Domestic pig
S. scrofa L. 1758	Wild boar
Equus caballus L. 1758	Horse
Macropus (*Thylogale*) *coxeni* Gray	Cape York wallaby
Phascolomys ursinus (Shaw)	Wombat
Hydrochoerus hydrochoerus (L. 1758)	Capybara
Taxidea taxus (Schreber)	Badger
Isothrix bistriatus bistriatus Wagner, 1845	
Rupicapra rupicapra (L. 1758)	Chamois
Euphractus sexcinctus L. 1758	Six-banded armadillo
Smutsia temminckii Smuts, 1832	Scaly ant-bear
Perameles nasuta Geoffroy, 1804	Long-nosed bandicoot
Didelphys aurita Wiedemann, 1826	Opossum
D. elegans	Opossum
D. marsupialis L. 1758	Common opossum
Kobus defassa ugandae Neumann, 1905	Waterbuck
Procavia capensis Pallas, 1766	Cape dassie
Pseudocheirus convoluta Oken, 1816	Ring-tailed possum
Thalacomys lagotis Reid, 1837	Rabbit bandicoot
Piranga erythromelas Vieillot	Scarlet tanager
Fringilla coelebs L. 1758	Chaffinch
Passer domesticus L. 1758	House sparrow
Alauda arvensis L. 1758	Skylark
Lanius nigriceps longicaudatus Gould, 1859	Shrike
Cinclus cinclus aquaticus Bechstein, 1803	Dipper
Otocoris alpestris merrelli	Horned lark
Erithacus luscinia (L. 1758)	
Sylvia atricapilla (L. 1758)	Blackcap
Hirundo rustica L. 1758	Common swallow
Delichon urbica urbica (L. 1758)	House martin
Riparia riparia (L. 1758)	Sand martin
Progne subis subis (L. 1758)	Martin
Colaptes cafer (Gmelin, 1790)	Woodpecker
Dendropicus cardinalis (Gmelin, 1788)	Woodpecker
D. fuscescens laepidus Cabani & Heine, 1863	Woodpecker
D. major (L. 1758)	Great spotted woodpecker
D. medius (L. 1758)	Lesser spotted woodpecker
Picus canus	Grey-headed woodpecker
Mesopicos ruwenzorii Sharpe, 1902	
Colius striatus (Gmelin, 1789)	Mouse bird
Cypselus affinis Hardwick, 1831	Swift
C. obvia	Swift
Caprimulgus europaeus L. 1758	Nightjar
Merops apiaster L. 1758	Common bee-eater
Podargus strigoides	Frogmouth
Otus asio asio (L. 1758)	American scops owl
Anser anser L. 1758	Grey lag goose

Somateria mollisima (L. 1758)	Eider duck
Calidris maritima (Brunnich, 1764)	Purple sandpiper
Charadrius hiaticula (L. 1758)	Ringed plover
Arenaria interores (L. 1758)	Turnstone
Sterna maxima Boddaert	Royal tern
Fulica americana Gmelin	Coot
Craspidophora magnifica (Viell. 1819)	
Columba domestica	Pigeon
Phasianus colchicus L. 1758	Pheasant
Gallus domesticus L. 1758	Domestic fowl
Procnias alba (Hermann, 1783)	
Nucifraga caryocatactes (L. 1758)	Thick-billed nutcracker
Merula nigra	
Crossarchus fasciatus	
Sericotes holosericeus	
Coluber flagellum flavigularis (Hallowell, 1852)	Coach-whip snake
Coluber viridiflavus Lacepede, 1789	
Chironius carinatus (L. 1754)	Wood snake
Elaphe guttata (L. 1766)	Corn snake
E. obsoleta obsoleta (Say, 1823)	Pilot black snake
E. quadrivittata quadrivittata (Holbrook, 1836)	Chicken snake
E. radiata (Schlegel, 1837)	Copper-headed rat snake
Pithuophis melanoleucus (Daudin, 1803)	Pine snake
P. sayi sayi (Schlegel, 1837) Cope, 1898	Bull snake
Thamnophis ordinoides ordinoides (Baird & Girard, 1852)	Cooper's snake
T. sirtalis sirtalis (L. 1758)	American garter snake
Lampropeltis calligaster (Harlan, 1827)	King snake
L. getulus getulus (L. 1766)	King snake
Heterodon contortrix contortrix (L. 1766)	Hog-nose snake
Natrix sipedon pictiventris Cope, 1895	Florida water snake
Erythrolamprus aesculapii (L. 1758)	Euscalapian snake
Drymarchon corais melanurus Dum. & Bibr. 1854	Gopher snake
Pseudoboa rustica (Cope, 1877)	
Ophis merremii Wagler, 1824	Merrem's boipeva snake
Crotalus atrox (Baird & Girard, 1853)	Western diamond rattle
C. durissus durissus L. 1758	Central American rattle
Dendroaspis angusticeps (Smith, 1849)	Green mamba
Lacerta agilis L. 1758	Sand lizard
L. viridis viridis (Laur. 1768)	Green lizard
Sceloporus graciosus graciosus (Baird & Girard, 1852)	Lizard
S. spinosus floridanus (Baird, 1858)	Lizard
Scaptira suborbitalis (Peters, 1869)	Spotted sand lizard
Mabuia quinquetaeniatus (Lichtenstein, 1823)	Skink
Tamandua tetradactyla (L. 1758)	
Pachydactylus bibronii Smith, 1846	Bibron's gecko
P. maculatus maculatus Gray, 1845	Spotted gecko
P. namaquensis (Sclater, 1899)	Namaqua gecko
Phyllodactylus porphyreus porphyreus (Daudin, 1802)	Marbled gecko
Homopholis wahlbergii (Smith, 1849)	Wahlberg's gecko
Hoplodactylus duvaucelli (Dum. & Bibr. 1836)	
Hemidactylus brooki Gray, 1845	House gecko
H. frenatus Dum. & Bibr. 1836	House gecko
H. leschenaulti Dum. & Bibr. 1836	Leschenault's gecko
H. tasmani Hewitt, 1932	Tasman's tropical gecko
H. turcicus turcicus (L. 1758)	Warty gecko
Tarentola mauritanica mauritanica (L. 1758)	Moorish wall gecko
Lygodactylus capensis capensis (Smith, 1849)	Cape gecko
Gymnodactylus louisiadensis De Vis, 1892	

Gymnodactylus pulchellus (Gray, 1828)	
G. russovi Stranch, 1887	
Afroedura karrooica wilmoti (Hewitt, 1926)	
A. transvaalica platyceps (Hewitt, 1925)	
Platyurus platurus (Schneid, 1792)	
Agama agama agama (L. 1758)	Spiny agama
A. atra Daudin, 1802	South African rock agama
A. hispida aculeata Merrem, 1820	Kalahari spiny agama
A. hispida armata Peters, 1854	Peter's spiny agama
A. hispida brachyura Boulanger, 1885	Namaqua spiny agama
A. mossambica mossambica Peters, 1854	Mozambique agama
A. nupta de Filippi, 1843	
A. stellio stellio (L. 1758)	Rough-tailed agama
Peropus oceanica (Lesson, 1830)	
Hemiphyllodactylus yunnanensis (Boulanger, 1903)	
Pristurus rupestris Blandford, 1874	Gecko
Gerrhosaurus flavigularis flavigularis Wiegmann, 1828	Yellow-throated plated lizard
G. validus validus Smith, 1849	Smith's plated rock lizard
Pseudocordylus microlepidotus microlepidotus (Cuvier, 1829)	Leathery crag lizard
P. subviridis subviridis Smith, 1838	Lesser Caiman lizard
Rhoptropus barnardi Hewitt, 1926	
Rhoptropella ocellata (Boulanger, 1885)	
Platysaurus capensis Smith, 1844	Cape red-tailed rock lizard
P. guttatus guttatus Smith, 1849	Transvaal red-tailed rock lizard
P. minor minor Fitz Simons, 1930	Lesser red-tailed rock lizard
P. wilhelmi Hewitt, 1909	Wilhelm's red-tailed rock lizard
Cordylus capensis robertsi (van Dam, 1921)	Roberts' girdled lizard
C. coeruleopunctatus (Meth. & Hewitt, 1919)	Blue spotted girdled lizard
C. cordylus cordylus (L. 1758)	Cape girdled lizard
C. namaquensis (Meth. & Hewitt, 1914)	Namaqua girdled lizard
C. polyzonus Smith, 1838	Karroo girdled lizard
C. vandami vandami (Fitz Simons, 1930)	Van Dam's girdled lizard
C. vittifer (Reichenow, 1887)	Transvaal girdled lizard
C. warreni warreni (Boulanger, 1908)	Warren's girdled lizard

REFERENCES

ALLMAN, G. J. (1847). Description of a new genus and species of Tracheary Arachnidans. *Ann. Mag. Nat. Hist.* **20**, 47–52.

AUGUSTSON, G. F. (1945). A new genus and new species of Dermanyssid mite (Acarina) from Texas. *Bull. S. Calif. Acad. Sci.* **44**, 46–8.

BANKS, N. (1899). A new species of the genus *Halarachne*. *Proc. Ent. Soc. Wash.* **4**, 212–14.

BANKS, N. (1901). A new genus of endoparasitic Acarians. *Geneesk. Tijdschr. Ned.-Indie*, **41**, 330–4.

BANKS, N. (1904). *A treatise on the Acarina, or mites. Proc. U.S. Nat. Mus.* **28**, 1–114.

BANKS, N. (1905). Descriptions of some new mites. *Proc. Ent. Soc. Wash.* **7**, 133–42.

BANKS, N. (1909). New Canadian mites. *Proc. Ent. Soc. Wash.* **11**, 133–43.

BANKS, N. (1910). New American mites. *Proc. Ent. Soc. Wash.* **12**, 2–12.

BERLESE, A. (1882–92). *Acari, Myriopoda et Scorpiones hucusque in Italia reperta.* Patavii, Florentiae.

BERLESE, A. (1888). Acari Austro Americani. *Bull. Soc. ent. ital.* **20**, 171–224.

BERLESE, A. (1903*a*). Acari Nuovi. Manipulus I. *Redia*, **1**, 260.

BERLESE, A. (1930*b*). Diagnosi di alcune nuove specie di Acari italiani Mirmecofili e liberi. *Zool. Anz.* **27**, 14.

BERLESE, A. (1906). Monographia del genera *Gamasus* Latr. *Redia*, **3**, 66–304.

BERLESE, A. (1910). Acari Nuovi. *Redia*, **6**, 199–234.

BERLESE, A. (1911). Acarorum species novae quindecim. *Redia*, **7**, 429–35.

BERLESE, A. (1917). Centuria seconda di Acari nuovi. *Redia*, **12**, 125–77.

BERLESE, A. (1918). Centuria quarta di Acari nuovi. *Redia*, **13**, 115–90.

BERLESE, A. (1921). Centuria quinta di Acari nuovi. *Redia*, **14**, 143–95.

BERLESE, A. & TROUESSART, E. L. (1889). Diagnoses d'Acariens nouveaux et peu connus. *Bull. Bibl. Sci. l'Ouest*, **9**, 121–43.

BERTHOLD, A. A. (1845). Beulemilbe. *Lehrb. Zool. Gottingen*, pp. 349–53.

BLANCHARD, R. (1906). Présence des Acariens du genre *Halarachne* chez les Phoques de l'Océan Antarctique. *Arch. Parasit., Paris*, **10**, 313.

CANESTRINI, G. & FANZAGO, F. (1877). Intorno agli Acari italiani. *Atti Ist. veneto*, **4** (5), 69–208.

CANESTRINI, G. & R. (1882). I Gamasi Italiani. Monografia. *Atti. Soc. Veneto-Trent.*

CANESTRINI, G. (1885). Prospetto dell' Acarofauna italiana. *Atti. Soc. Veneto-Trent.*

CANESTRINI, G. (1895). Ueber einen neuen Parasiten der Saugethiere. *Zool. Anz.* **18**, 114–15.

CHANDLER, W. L. & RUHE, D. S. (1940). *Pneumonyssus caninum* n.sp. a mite from the frontal sinus of the dog. *J. Parasit.* **26**, 59–70.

CLAPAREDE, E. (1868). Studien an Acariden. *Z. wiss. Zool.* **18**, 445–546.

COOREMAN, J. (1946). *Rhinoecius oti*, n.gen., n.sp. (Acarien, Rhinonyssinae). *Bull. Mus. Hist. nat. Belg.* **22**, 9, 1–4.

CSOKOR, J. (1897). Über Haarsackmilben und eine neue Varietät derselben bei Schweinen, *Demodex phylloides. Verh. zool.-bot. Ges. Wien*, **29**, 419–50.

DEGEER, C. (1778). *Mémoires pour servir à l'histoire des Insectes*, **7**, 94.

DOETSCHMAN, W. H. (1944). A new species of endoparasitic mite of the family Halarachnidae (Acarina). *Trans. Amer. Micr. Soc.* **63**, 68–72.

DUGES, A. (1834). Nouvelles observations sur les Acariens. *Ann. Sci. nat. (zool.)*, **11**, 104–6.

EHLERS, E. (1873). Die Kratzmilben der Vögel. Ein Beitrag zur Kenntniss der Sarcoptiden. *Z. wiss. Zool.* **23**, 228–53.

EWING, H. E. (1910). New Acarina from India. *Trans. Acad. Sci. St Louis*, **19**, 113–21.

EWING, H. E. (1922). Dermanyssid mites of N. America. *Proc. U.S. Nat. Mus.* **62**, no. 13, 1–26.

EWING, H. E. (1924*a*). A new mite from the lung of a rattlesnake. *Proc. Ent. Soc. Wash.* **26**, 179.

EWING, H. E. (1924*b*). Ectoparasites of some Polynesian and Malaysian rats of the genus *Rattus*. *Bull. Bish. Mus. Honolulu*, **14**, 7–11.

EWING, H. E. (1925*a*). New mites of the family Dermanyssidae. *Ent. News*, **36**, 18–22.

EWING, H. E. (1925*b*). New mites of the parasitic genus *Haemogamasus* Berlese. *Proc. Biol. Soc. Wash.* **38**, 138–43.

EWING, H. E. (1925*c*). New parasitic mites of the genus *Laelaps*. *Proc. Ent. Soc. Wash.* **27**, no. 1, 1–7.

EWING, H. E. (1929*a*). *Manual of External Parasites.* Baltimore: C. Thomas.

EWING, H. E. (1929*b*). Notes on the lung mites of Primates (Acarina: Dermanyssidae) including the description of a new species. *Proc. Ent. Soc. Wash.* **31**, 126–30.

EWING, H. E. (1933*a*). New genera and species of parasitic mites of the superfamily Parasitoidea. *Proc. U.S. Nat. Mus.* **82**, no. 30, 1–14.

EWING, H. E. (1933*b*). A new pit-producing mite from the scales of a South American snake. *J. Parasit.* **20**, no. 1, 53–6.

EWING, H. E. (1936). A short synopsis of the North American species of the mite genus *Dermanyssus*. *Proc. Ent. Soc. Wash.* **38**, 47–54.

EWING, H. E. (1938). North American mites of the subfamily Myobiinae, new subfamily (Arachnida). *Proc. Ent. Soc. Wash.* **40**, no. 1, 180–97.

EWING, H. E. & BAKER, E. W. (1947). *Myonyssus jamesoni*, a new Liponyssid mite (Acarina: Laelaptidae) from *Blarina brevicauda* (Say). *J. Parasit.* **33**, no. 4, 376–9.

EWING, H. E. & STOVER, A. J. (1915). New parasitic mites (Acarina). *Ent. News*, **26**, 109–14.

EYNDHOVEN, G. L. VAN (1940). Ein neuer Fledermausparasit *Labidocarpus belsorum* nov.spec. (Acar.). *Zool. Anz.* **130**, 9/10, 230–4.

EYNDHOVEN, G. L. VAN (1946). Diagnoses of two epizootic mites. (Chiropterological notes, no. 24). *Ent. Ber., Amst.*, **12**, 267/268, 30–31.

FAHRENHOLZ, H. (1908). Aus dem Myobien Nachlass des Herrn Poppe. *Abh. naturw. Ver. Bremen*, **19**, 3, 359–70.

FERRIS, G. F. (1925). On the species of the genus *Halarachne* (Acarina: Gamasidae). *Parasitology*, **17**, 163–7.

FERRIS, G. F. (1932). Ectoparasites of Marquesan rats. *Bull. Bish. Mus. Honolulu*, **98**, 117–27.

FINNEGAN, S. (1934). On a new species of mite of the family Halarachnidae from the Southern sea lion. '*Discovery*' *Rep.* **7**, 319–28.

FLOCH, H. & ABONNENC, E. (1944). Description d'un acarien nouveau: *Geckobia guyanensis* n.sp. (Fam. Pterygosomatidae) parasite d'un platydactyle. *Publ. Inst. Pasteur Guyane*, **93**, 1–9.

FONSECA, F. DA (1932). Notas de Acareologia. *Mem. Inst. Butantan.* **7**, 135–46.

FONSECA, F. DA (1934). Der Schlangenparasit *Ixobioides butantanensis* n.gen., n.sp. *Z. Parasitenk.* **6**, 4, 508–27.

FONSECA, F. DA (1935*a*). New Genera and Species of Acari 'Laelaptidae' from Brazilian Rodents. *XII Congres Int. zool. Lisbon*, pp. 1597-1615.

FONSECA, F. DA (1935*b*). Notas de Acareologia. *Mem. Inst. Butantan.* **9**, 33–8, 43–72 and 89–97.

FONSECA, F. DA (1936). Notas de Acareologia. *Mem. Inst. Butantan.* **10**, 43–6, 51–9.

FONSECA, F. DA (1938). Notas de Acareologia. *Mem. Inst. Butantan.* **12**, 7–53, 103–23 and 147–54.

FONSECA, F. DA (1939*a*). Os Laelaptidae gigantes, parasitas de roedores sul americanos; genero e especies novos (Acari). *Mem. Inst. Butantan.* **12**, 7–53.

FONSECA, F. DA (1939*b*). Novos estudos sobre o genero *Laelaps* Koch, 1836 (Acari: Laelaptidae). *Mem. Inst. Butantan.* **12**, 103–23.

FONSECA, F. DA (1939*c*). *Liponissus brasiliensis* sp.n., parasita habitual de roedores e acidental do homen. *Mem. Inst. Butantan.* **12**, 147–54.

FONSECA, F. DA (1940*a*). Notas de Acareologia. *Mem. Inst. Butantan.* **14**, 53–64.

FONSECA, F. DA (1940*b*). Notas de Acareologia. *Rev. Ent. S. Paulo (Rio de J.)*, **11**, 104–19.

FONSECA, F. DA (1941*a*). Notas de Acareologia. *Mem. Inst. Butantan.* **15**, 103–18.

FONSECA, F. DA (1941*b*). Notas de Acareologia. *Ciencia Mexico*, **2**, 6/7, 262–5.

FONSECA, F. DA (1942). Notas de Acareologia. *Mem. Inst. Butantan.* **16**, 149–56.

FONSECA, F. DA (1948). A Monograph of the genera and species of Macronyssidae Oudemans, 1936 (Synon: Liponyssidae Vitzthum, 1931) (Acari). *Proc. zool. Soc. Lond.* **118**, no. 2, 249–334.

FOX, I. (1937). A new Cheyletid mite (Acarina) parasitic on the Caroline Jumping Mouse, *Zapus hudsonicus americanus* (Barton). *Proc. Ent. Soc. Wash.* **39**, 227–30.

FOX, I. (1946). A new genus *Borinquolaelaps*, and new species of mites from rats in Puerto Rico. *J. Parasit.* **32**, no. 5, 445–52.

FÜRSTENBERG, M. H. F. (1861). *Die Kratzmilben der Menschen und Thiere*. Leipzig.

GERVAIS, P. (1844). *Hist. Nat. Ins. Apt.* **3**, 133–288.

GIEBEL, C. (1871). Ueber einige Milben. *Z. Ges. Naturw.* **38**, 31–2.

GUNTHER, C. E. M. (1942*a*). New parasitid mites from New Guinea (Acarina: Parasitidae). *Proc. Linn. Soc. N.S.W.* **67**, nos. 1/2, 87–9.

GUNTHER, C. E. M. (1942*b*). Notes on the Listrophoridae (Acarina: Sarcoptoidea). *Proc. Linn. Soc. N.S.W.* **67**, nos. 3/4, 109–10.

HALLER, G. (1877). *Freyana* und *Picobia*, zwei neue Milbengattungen. *Z. wiss. Zoll.* **30**, 91.

HALLER, G. (1880). Zur Kenntnis der Tyroglyphiden und Verwandten. *Z. wiss. Zool.* **34**, 255–95.

HALLER, G. (1882). Beitrag zur Kenntnis der Milbenfauna Wurttembergs. *Jber. Ver. nat. Wirtt.* p. 312.

HALLER, G. (1884). Beschreibung einiger neuen Milben. *Arch. Naturgesch*, **1**, 217–36.

HEFLEY, H. M. (1935). A new mite from the common skunk: *Mephitis nigra*. *J. Kans. Ent. Soc.* **8**, no. 1, 22–4.

HERING (1838). Die Kratzmilben d. Thier u. e. verw. Arten. *Nova Acta Leop. Carol.* **18**, 2, 573–624.

HERMANN, J. F. (1804). *Mémoire Apterologique*. Strasbourg.

HEYDEN, C. H. G. VON (1826). Versuch einer Systematischen Eintheilung der Acariden. *Oken, Isis*, **19**, 608–13.

HIRST, S. (1912). On two new parasitic Acari of the genus *Leiognathus* Cn. *Bull. Ent. Res.* **3**, 369–72.

HIRST, S. (1913). On three new species of Gamasid mites found on rats. *Bull. Ent. Res.* **4**, 119–24.

HIRST, S. (1914*a*). Preliminary list of the Acari occurring on the brown rat (*Mus norvegicus*) in Great Britain, with the description of a new species (*Haemogamasus oudemansi*). *Bull. Ent. Res.* **5**, no. 2, 119–24.

HIRST, S. (1914*b*). On the parasitic Acari found on the species of rodents frequenting human habitations in Egypt. *Bull. Ent. Res.* **5**, 215–19.

HIRST, S. (1914*c*). Report on the Arachnida and Myriopoda collected by the British Ornithologists Union Expedition and the Wollaston Expedition in Dutch New Guinea. *Trans. Zool. Soc. Lond.* **20**, no. 10, 325–34.

HIRST, S. (1915*a*). On a blood-sucking Gamasid mite (*Ichoronyssus serpentium* sp.n.?) parasitic on Couper's snake. *Proc. Zool. Soc. Lond.* pp. 383–6.

HIRST, S. (1915*b*). On some new Acarine parasites of rats. *Bull. Ent. Res.* **6**, 183–90.

HIRST, S. (1916). Notes on Parasitic Acari. Descriptions of two new African mites of the family Gamasidae. *J. Zool. Res.* **1**, 76–81.

HIRST, S. (1917*a*). On some new mites of the suborder Prostigmata living on lizards. *Ann. Mag. Nat. Hist.* (8), **19**, 136–43.

HIRST, S. (1917*b*). On three new parasitic Acari. *Ann. Mag. Nat. Hist.* (8), **20**, 431–4.

HIRST, S. (1918). On four new species of the genus *Demodex* Owen. *Ann. Mag. Nat. Hist.* (9), **2**, 145–6.

HIRST, S. (1919). On two new parasitic mites (*Myocoptes intoni* and *Psoroptes natalensis*). *Ann. Mag. Nat. Hist.* (9), **3**, 524.

HIRST, S. (1921). On some new parasitic mites. *Proc. Zool. Soc. Lond.* pp. 769–802.

HIRST, S. (1923*a*). On three new species of *Laelaps* occurring on S. African rodents. *Ann. Mag. Nat. Hist.* (9), **12**, 690–2.

HIRST, S. (1923*b*). On some new or little known species of Acari. *Proc. Zool. Soc. Lond.* pp. 971–1000.

HIRST, S. (1925*a*). Descriptions of new Acari, mainly parasitic on Rodents. *Proc. Zool. Soc. Lond.* pp. 49–69.

HIRST, S. (1925*b*). Acarina (excl. Ixodidae) in Parasitologia Mauritanica. *Bull. Com. A.O.F.G. Senegal*, **8**, 95–100.

HIRST, S. (1926). On the parasitic mites of the suborder Prostigmata (Trombidioidea) found on lizards. *J. Linn. Soc. Zool.* **36**, 173–200.

HIRST, S. (1927). Note on Acari, mainly belonging to the genus *Spinturnix* von Heyden. *Proc. Zool. Soc. Lond.* pp. 323–38.

HIRST, S. (1931). On some new Australian Acari (Trombidiidae, Anystidae and Gamasidae). *Proc. Zool. Soc. Lond.* pp. 561–4.

HOFFMANN, A. M. (1944*a*). Un nuevo acaro parasito de murcielagos. *Ann. Inst. Biol., Mexico*, **15**, no. 1, 185–9.

HOFFMANN, A. M. (1944*b*). *Periglischrus vargasi* n.sp. (Acarina: Parasitidae). *Rev. Inst. Salubr. Enferm. trop. Mexico*, **5**, 2, 91–6.

HUBBARD, W. E. (1938). *Ophiopneumicola colubri* n.gen., n.sp., a lung mite from a snake. *Trans Amer. Micr. Soc.* **57**, 400-6.

HUBBARD, W. E. (1939). *Entonyssus ewingi* n.sp. an Ophidian lung mite. *Amer. Midl. Nat.* **21**, 657–62.

JAMESON, E. W. (1947). A new mite *Eubrachylaelaps crowei* from the grasshopper mouse, *Onychomys leucogaster arcticeps* (Rhoads). *J. Parasit.* **33**, no. 5, 391–2.

JAMESON, E. W. (1948). Myobiid mites (Acarina: Myobiinae) from Shrews (Mammalia: Soricidae) of Eastern North America. *J. Parasit.* **34**, no. 4, 336–42.

JAMESON, E. W. (1949). Myobiid mites (Acarina: Myobiidae) from *Condylura cristata* (Linnaeus) and *Neurotrichus gibbsii* (Baird) (Mammalia: Talpidae). *J. Parasit.* **35**, no. 3, 423–30.

KEEGAN, H. L. (1943). Three new Ophidian lung mites of the sub-family Entonyssinae Ewing, 1922. *Parasitology*, **35**, 128–33.

KEEGAN, H. L. (1946). Six new mites of the superfamily Parasitoidea. *Trans. Amer. Micr. Soc.* **65**, no. 1, 69–77.

KOCH, C. L. (1835–44). *Crustaceen, Myriopoden und Arachniden Deutschlands*. Regensburg.

KOCH, C. L. (1842). *Uebersicht des Arachnidensystems*. **3**.

KOLENATI, F. A. (1856). Parasiten der Chiroptern. *Vers. dtsch. naturf. Aertzte*. pp. 25–9.

KOLENATI, F. A. (1857). Synopsis prodroma der auf Chiroptern als Epizooen vorkommenden Lausmilben *Carida*, Kolenati. *Wien. ent. Mschr.* **1** (2), 4–7.

KOLENATI, F. A. (1858). Beitrag zur Kenntnis der Arachniden. *S.B. Akad. wiss. Wien*, **35**, 155–90.

LATREILLE, P. A. (1796). *Précis des caractères génériques des Insectes, etc.* Brive.

LAWRENCE, R. F. (1935). The prostigmatic mites of South African lizards. *Parasitology*, **27**, 1–45.

LAWRENCE, R. F. (1936). The prostigmatic mites of South African lizards. *Parasitology*, **28**, 1–39.

LAWRENCE, R. F. (1938). A new Acarine parasite of bats. *Parasitology*, **30**, 309–13.

LAWRENCE, R. F. (1939). A new mite from the South African Pangolin. *Parasitology*, **31**, 451–7.

LAWRENCE, R. F. (1944). A new parasitic mite from the Golden Mole. *Proc. Zool. Soc. Lond.* **114**, 302–6.

LAWRENCE, R. F. (1948*a*). Some new pilicolous mites from South African mammals. *Parasitology*, **39**, 39–42.

LAWRENCE, R. F. (1948*b*). Studies on some parasitic mites from Canada and South Africa. *J. Parasit.* **34**, no. 5, 364–79.

LEIDY, J. (1872). On a mite in the ear of the ox. *Proc. Acad. Nat. Sci. Philad.* pp. 9–10, 118.

LEYDIG, F. (1859). Ueber Haarsack und Kratzmilbe. *Arch. Naturgesch.* **25**, 345.

LINNAEUS, C. (1758). *Systema Naturae* (10th ed.), **1**, 615–18.

LJUNGH, S. I. (1799). *Nova Act. Reg. Soc. Sci. Uppsala*, **6**, 10.

LOMBARDINI, G. (1944). *Spinturnix vespertilionis* (L.) Duf. *Ianzai* v.n. *Redia*, **30**, 5.

MEGNIN, P. (1876). Mémoire sur l'organisation et la distribution zoologique des Acariens de la Famille des Gamasides. *J. Anat. Phys.* **12**, 228–336.

MEGNIN, P. (1877). Monographie de la Tribu des Sarcoptides Psoriques, qui comprend tous les Acariens de la gale de l'Homme et des Animaux. *Rev. zool.* (3), **5**, 46–213.

MEGNIN, P. (1878). Note sur un nouvel acarien parasite (*Geckobia latasti*). *Bull. Soc. ent. Fr.* **5**, 8.

MEGNIN, P. (1879). Les Acariens parasites du tissu cellulaire et des reservoirs aériens chez les oiseaux. *J. Anat. Phys.* **15**, 123–53.

MEGNIN, P. (1880). *Les Parasites et les Maladies Parasitaires*. 478 pp., 26 pls. and 63 text-figs. Paris.

MEGNIN, P. (1884). Étude sur l'*Ophionyssus natricis* P. Gervais. *Bull. Soc. zool. Fr.* **9**, 107–13.

MEILLON, B. DE & LAVOIPIERRE, M. (1944). New records and species of biting insects from the Ethiopian region. *J. Ent. Soc. S. Afr.* **7**, 38–67.

MICHAEL, A. D. (1844). On an undescribed Acarus of the genus *Myobia*. *J. Quekett Micr. Cl.* (2), **2**, 1–7.

MICHAEL, A. D. (1889). On some unrecorded parasitic Acari found in Great Britain. *J. Linn. Soc. Lond.* **20**, 400–6.

MICHAEL, A. D. (1892). On the variations in the internal anatomy of the Gamasinae. *Trans. Linn. Soc. Lond.* **5**, 31–7.

NEUMANN, L. G. (1902). *Spelaeorhynchus praecursor* n.gen., n.sp. Nouvel Acarien parasite. *Arch. parasit.* **5**, 31–7.

NEWELL, I. M. (1947). Studies on the Morphology and Systematics of the family Halarachnidae Oudemans, 1906 (Acarina: Parasitoidea). *Bull. Bingham Oceanogr. Coll.* **10**, nos. 3/4, 235–66.

NEWSTEAD, R. & TODD, J. D. (1906). On a new Dermanyssid Acarid. *Mem. Lpool Sch. Trop. Med.* **18**, 41–4.

NITZSCH, C. L. (1818). Acarina in Ersch & Gruber. *Allg. Encycl. Wiss.* **1**, 246–52.

OUDEMANS, A. C. (1897). *Tijdschr. Ent.* **40**, 49–50.

OUDEMANS, A. C. (1898). A Sarcoptes of a bat, *Nycteridocoptes Poppei* n.g., n.sp. *Tijdschr. Ent.* **40**, nos. 3/4, 270.

OUDEMANS, A. C. (1901). Notes on Acari. Third Series. *Tijdschr. ned. dierk. Ver.* **7**, 50–88.

OUDEMANS, A. C. (1902*a*). Acarologische Aanteekeningen. 6. *Ent. Ber., Amst.*, **6**, 36.

OUDEMANS, A. C. (1902*b*). Notes on Acari. Fourth Series. *Tijdschr. ned. dierk. Ver.* (2), **7**, nos. 3/4, 276–311.

OUDEMANS, A. C. (1904*a*). Notes on Acari. Eleventh Series. *Tijdschr. Ent.* **46**, 93–134.

OUDEMANS, A. C. (1904*b*). *Laelaps versteegii*, a new species of parasitic mite. *Notes Leyden Mus.* **24**, no. 9, 223–31.

OUDEMANS, A. C. (1906). Acarologische Aanteekeningen. 22. *Ent. Ber., Amst.*, **28**, 55–62.

OUDEMANS, A. C. (1910). Acarologische Aanteekeningen. 32. *Ent. Ber., Amst.*, **53**, 67–74.

OUDEMANS, A. C. (1912). Acarologische Aanteekeningen. 39. *Ent. Ber., Amst.*, **3**, 63, 215–17.

OUDEMANS, A. C. (1914). Acarologische Aanteekeningen. 52. *Ent. Ber., Amst.*, **4**, 76, 65–73.

OUDEMANS, A. C. (1916). Acarologische Aanteekeningen. 60. *Ent. Ber., Amst.*, **4**, 91, 308–16.

OUDEMANS, A. C. (1925). Acarologische Aanteekeningen. 79. *Ent. Ber., Amst.*, **7**, 146, 26–34.

OUDEMANS, A. C. (1928). Acarologische Aanteekeningen. 94. *Ent. Ber., Amst.*, **7**, 164, 374–82.

OUDEMANS, A. C. (1929). Acarologische Aanteekeningen. 99. *Ent. Ber., Amst.*, **8**, 169, 11–20.

OUDEMANS, A. C. (1935). Kritische Literaturübersicht zur Gattung *Pneumonyssus*. Beschreibung dreier Arten darunter einer neuen. *Z. Parasitenk.* **7**, 4, 466–512.

OUDEMANS, A. C. (1939). Neue Funde auf dem Gebiete der Systematik und der Nomenclatur der Acari. *Zool. Anz.* **126**, 303–9.

PAGENSTECHER, H. A. (1861*a*). *Listrophorus leuckarti*, ein neues Milbengeschlecht. *Siebold Kolliker. Zool.* **11**, 109–19.

PAGENSTECHER, H. A. (1861*b*). *Listrophorus gibbus*, nebst nachträglichen Bemerkungen über *Listrophorus leuckarti*. *Siebold Kolliker. Zool.* **11**, 151–61.

PEARSE, A. S. (1929). Two new mites from the gills of land crabs. *Pap. Tortugas. Lab.* **26**, 225–30.

PEARSE, A. S. (1930). Parasites of Crustacea. *Ann. zool. Japan.* **13**, 1–8.

PERKINS, M. (1925). On *Acanthophthirius etheldredae*, a new genus and species of Anoplura from the Pipistrelle bat. *Ann. Mag. Nat. Hist.* (9), **16**, 175–8.

PETERS, W. (1849). Eine neue Acarine Gattung *Pterygosoma*. *S.B. ges. naturf. Fr. Berl.* **19**, 6.

POPPE, S. A. (1887). Über parasitische Milben. *Abh. naturw. Ver. Bremen*, **10**, 1, 205.

POPPE, S. A. (1896). Beitrag zur Kenntnis der Gattung *Myobia* v. Heyden. *Zool. Anz.* **19**, 508/9, 327–33 and 337–49.

POPPE, S. A. & TROUESSART, E. L. (1895). Sur les métamorphoses du genre *Myobia* et diagnoses d'espèces nouvelles d'Acariens. *Bull. Soc. Ent. Fr.* **8**, 84.

RADFORD, C. D. (1934). Notes on mites of the genus *Myobia*. Pt. I. *Northw. Nat.* **9**, 356–64.

RADFORD, C. D. (1935). Notes on mites of the genus *Myobia*. Pt. II. *Northw. Nat.* **10**, 248–58.

RADFORD, C. D. (1936*a*). Notes on mites of the genus *Myobia*. Pt. III. *Northw. Nat.* **11**, 34–9.

RADFORD, C. D. (1936*b*). Notes on mites of the genus *Myobia*. Pt. IV. *Northw. Nat.* **11**, 144–51.

RADFORD, C. D. (1937). A new species of mite of the genus *Entonyssus* Ewing. *Northw. Nat.* **12**, 38–42.

RADFORD, C. D. (1938*a*). *Pneumonyssus procavians* n.sp. a new lung mite. *Ann. Transv. Mus.* **19** (2), 147–52.

RADFORD, C. D. (1938*b*). Notes on some new species of parasitic mites. *Parasitology*, **30**, 427–40.

RADFORD, C. D. (1939). Notes on some new species of parasitic mites. Pt. 2. *Parasitology*, **31**, 241–54.

RADFORD, C. D. (1940). Notes on some new species of parasitic mites. Pt. 3. *Parasitology*, **32**, 91–104.

RADFORD, C. D. (1941). Notes on some new species of parasitic mites. Pt. 4. *Parasitology*, **33**, 306–15.

RADFORD, C. D. (1942*a*). New ectoparasitic mites (Acarina) from Uganda. *Parasitology*, **34**, 185–94.

RADFORD, C. D. (1942*b*). New parasitic mites (Acarina). *Parasitology*, **34**, 294–307.

RADFORD, C. D. (1943). Genera and species of parasitic mites (Acarina). *Parasitology*, **35**, 58–81.

RADFORD, C. D. (1944). New parasitic mites (Acarina) from rodents. *Parasitology*, **35**, 161–6.

RADFORD, C. D. (1947*a*). Parasitic mites from snakes and rodents. (Acarina, Cheyletidae, Listrophoridae and Laelaptidae). *Proc. Zool. Soc. Lond.* **117**, 228–40.

RADFORD, C. D. (1947*b*). Two new bat mites of the genus *Ancystropus* (Acarina: Spinturnicidae). *Proc. Zool. Soc. Lond.* **117**, 305–12.

RADFORD, C. D. (1948*a*). New parasitic mites (Acarina: Myialgesidae and Listrophoridae). *Proc. Zool. Soc. Lond.* **118**, 933–7.

RADFORD, C. D. (1948*b*). A revision of the fur mites Myobiidae (Acarina). *Bull. Mus. Hist. nat. Paris*, **20** (2), 5, 458–64; 6, 525–31 *et seq.*

RAILLIET, A. (1895). *Traité de Zoologie Médicale et Agricole*, 2nd ed.

REDI, F. (1674). *Esperionze intorno all Generazione degl' Insetti, etc.* Firenze.

RODHAIN, J. (1921). Un Sarcoptide, nouveau parasite de la Roussette Africaine (*Eidolon helvum* Kerr). *C.R. Soc. Biol., Paris*, **84**, 757–9.

RODHAIN, J. (1923). Deux Sarcoptides psoriques parasites des Roussettes Africaine au Congo. *Rev. zool. afr.* **11**, 17–39.

SAMBON, L. W. (1928). *Ophioptes parkeri*. A new species and genus of Cheyletid inhabiting the scales of reptiles. *Ann. Trop. Med. Parasit.* **22**, no. 1, 137–42.

SCHMIDT, F. L. (1940). *Entonyssus vitzthumi* (Acarina) a new Ophidian lung mite. *J. Parasit.* **26**, 309–13.

SCHRANK, F. P. VON (1781). *Pediculus muris musculi. Enum. Insect. Aust. indig.* pp. 501–2.

SELLNICK, M. (1941). Milben von Fernando Poo. *Zool. Anz.* **136**, 11/12, 221–8.

SELLNICK, M. (1944). Eine neue parasitische Milbe von *Epimys norvegicus* Erxl. *Z. Parasitenk.* **13**, 248–53.

STRANDTMANN, R. W. (1941). A new species of mite, *Ichoronyssus dentipes* (Acarina: Liponyssinae), from the Cotton Rat. *J. Parasit.* **33**, no. 1, 51–6.

STRANDTMANN, R. W. (1947). *Atricholaelaps megaventralis*, a new species of parasitic mite. (Acarina: Laelaptidae). *Ent. Soc. Wash.* **49**, no. 4, 112–14.

STRANDTMANN, R. W. (1948*a*). *Atricholaelaps traubi* and *A. chinchillulae* from Callosciurius and the Chinchilla respectively. *Proc. Ent. Soc. Wash.* **50**, no. 7, 187–92.

STRANDTMANN, R. W. (1948*b*). The Mesostigmatic nasal mites of birds. I. Two new genera from shore and marsh birds. *J. Parasit.* **34**, no. 6, 505–14.

TIRABOSCHI, C. (1904). Les Rats, les Souris, et leurs parasites cutanes, dans leurs rapportes avec le propagation de la peste bubonique. *Arch. Parasit., Paris*, **8**, 337–8.

TRAGARDH, I. (1904). Monographie der arktischen Acariden. *Fauna arctica*, p. 41. Jena.

TRAGARDH, I. (1905). Acariden aus Ägypten und der Sudan. *Swed. Zool. exp. Egypt White Nile*, **2**, 1–124.

TRAGARDH, I. (1908). *Kilimandjaro-Meru Expedition, Uppsala*, **20**, no. 3, 54–7.

TRAGARDH, I. (1931). Acarina from the Juan Fernandez Islands. *Nat. Hist. Juan Fernandez and Easter Islands, Uppsala*, **3**, 553–628.

TROUESSART, E. L. (1893). Note sur les Sarcoptides pilicoles (Listrophorinae). *C.R. Soc. Biol., Paris*, **45**, 698–700.

TROUESSART, E. L. (1894). Note sur les Acariens parasites des fosses nasales des oiseaux. *C.R. Soc. Biol., Paris*, **1** (10), 723–31.

TROUESSART, E. L. (1895*a*). Note sur un Acarien parasite des fosses nasales de l'Oie domestique. *Rev. Sci. Nat. app. Bull. Soc. nat. d'Acclimatation*, **42**, 392–4.

TROUESSART, E. L. (1895*b*). Sur les métamorphoses du genre *Myobia* et diagnoses d'espèces nouvelles d'Acariens. *Bull. Soc. Ent.* **8**, 84.

TROUESSART, E. L. (1895*c*). Description d'un genre nouveau (*Labidocarpus*) et de deux nouvelles de Sarcoptides pilicoles, avec figures. *Bull. Soc. ent. France, Congr. Ann.* pp. 38–9, 82–7.

TROUESSART, E. L. (1896*a*). Genre nouveau et espèce nouvelle de Sarcoptides pilicoles (Chirodiscinae) (Acariens: Arach.). *Bull. Soc. ent. Fr.* pp. 27–9.

TROUESSART, E. L. (1896*b*). Description du *Schizocarpus mingaudi* (Arachn) nouveau Sarcoptide pilicole vivant sur le Castor. *Bull. Soc. ent. Fr.* pp. 91–7.

TROUESSART, E. L. (1896*c*). Sur un nouveau type de Sarcoptides pilicoles (*Schizocarpus mingaudi* g. et sp.n) vivant sur le Castor. *C.R. Soc. Biol., Paris*, **48**, 109–10.

TROUESSART, E. L. (1902). Existence de la Parthénogenèse chez le '*Gamasus auris*' Leidy de l'oreille du Bœuf domestique. *C.R. Soc. Biol., Paris*, **54**, 1335–7.

TROUESSART, E. L. (1904). *Leiognathus blanchardi* sp.n. Acarien parasite de la Marmotte des Alpes. *Arch. Parasit., Paris*, **8**, 558–61.

TROUESSART, E. L. (1917). Troisième note sur les Sarcoptides pilicoles et description de genres nouveaux. *Bull. Soc. zool. Fr.* **42**, 151–8.

TROUESSART, E. L. & NEUMANN, G. (1890). Un type nouveau de Sarcoptides plumicoles le *Chirodiscus amplexans* g.n., sp.n. *Bull. sci. Fr. Belg.* **22**, 392–8.

TUBB, J. A. (1937). Arachnida in Report of the Expedition of the McCoy Society for Field Investigation and Research (Lady Julia Percy Island). *Proc. Roy. Soc. Vict.* N.S. **49**, no. 2, 412–21.

TURK, F. A. (1945*a*). Studies of Acari—Second Series. Descriptions of new species and notes on established forms of parasitic mites. *Parasitology*, **36**, 133–41.

TURK, F. A. (1945*b*). Studies of Acari. V. Notes on and descriptions of new and little-known British Acari. *Ann. Mag. Nat. Hist.* (II), **12**, 785–820.

TURK, F. A. (1946). A new genus and two new species of mites parasitic on Muridae. *Ann. Mag. Nat. Hist.* (II), **13**, 347–54.

TURK, F. A. (1947). Studies of Acari. IV. A Review of the lung mites of snakes. *Parasitology*, **38**, nos. 1/2, 17–26.

TURK, F. A. (1948). Insecticolous Acari from Trinidad, B.W.I. *Proc. Zool. Soc. Lond.* **118**, 82–125.

TYRRELL, J. B. (1883). On the occurrence in Canada of two species of parasitic mites. *Proc. Canad. Inst.* **1**, 332–43.

ULLRICH, H. (1939). Über einige neue Schmarotzermilben dei Elch, Biber und Schneehase. *Z. Parasitenk.* **10**, 553–8.

VAIL, E. L. & AUGUSTSON, G. F. (1943). A new ectoparasite (Acarina: Cheyletidae) from domestic rabbits. (*Ewingella* gen.nov. *americana* sp.n.) *J. Parasit.* **29**, no. 6, 419–21.

VITZTHUM, H. GRAF (1914). Beschreibung einiger neuen Milben. *Zool. Anz.* **44**, 323.

VITZTHUM, H. GRAF (1918). Acarologische Beobachtungen. 2 Reihe. *Arch. Naturgesch.* **84**, 1–40.

VITZTHUM, H. GRAF (1920). Acarologische Beobachtungen. 4 Reihe. *Arch. Naturgesch.* **86**, 1–69.

VITZTHUM, H. GRAF (1924). Acarologische Beobachtungen. 8 Reihe. *Arch. Naturgesch.* **90**, 10, 1–86.

VITZTHUM, H. GRAF (1925). Die unterirdische Acarofauna. *Jena Z. Naturw.* **62**, 125–86.

VITZTHUM, H. GRAF (1926). Malayische Acari. *Treubia*, **8**, 1, 1–198.

VITZTHUM, H. GRAF (1928*a*). Acarologische Beobachtungen. 12 Reihe. *Zool. Anz.* **75**, 181–98.

VITZTHUM, H. GRAF (1928*b*). Acarologische Beobachtungen. 13 Reihe. *Zool. Anz.* **75**, 281–95.

VITZTHUM, H. GRAF (1930*a*). Milben als Pestträger. *Zool. Jb.* **60**, 381–428.

VITZTHUM, H. GRAF (1930*b*). *Pneumonyssus stammeri*, ein neuer Lungenparasit. *Z. Parasitenk.* **2**, 595–615.

VITZTHUM, H. GRAF (1930c). Acarologische Beobachtungen. 14 Reihe. *Zool. Jb.* **59**, 2/3, 281–350.

VITZTHUM, H. GRAF (1931*a*). Neu parasitische Fledermausmilben aus Venezuela. *Z. Parasitenk.* **4**. 1–47.

VITZTHUM, H. GRAF (1931*b*). In Kukenthals, *Handbuch der Zoologie*, **3**, 2, 1–160.

VITZTHUM, H. GRAF (1935*a*). Über die Gattung *Entonyssus* Ewing (Acari). *Z. Parasitenk.* **7**, 709–16.

VITZTHUM, H. GRAF (1935*b*). Milben aus der Nasenhöhle von Vögeln. *J. Ornith.* **83**, 563–87.

VITZTHUM, H. GRAF (1941). Acarina in Bronn's *Klassen und Ordnungen des Tierreichs*, **5**, 5, Lfg. 1–7, 1–1100.

VIZIOLI, F. (1868). Osservazioni microscopiche sopra alcuni noduli sottocutanei de' polli e descrizione di un nuovo genere di Acaridei parasita degli uccelli. (*Sarcoptes cisticola* n.g. et n.sp.) *Giorn. anat. Fisiol.* **1**, 257–72.

VOROBIOV, M. M. (1938). Description of the morphology and biology of *List* (r) *ophorus larisi* n.sp. found on white mice. *Actes Sci. Inst. veter. Kiev*, **1**, 151–7.

WALKENAER, C. A. (1847). *Histoire Naturelle des Insectes, Aptères*, **4**, 346–54. Paris.

WEIDMAN, F. D. (1916). *Cytoleichus penrosei*, a new arachnoid parasite found in the diseased lungs of a prairie dog, *Cynomys ludovicianus*. *J. Parasit.* **3**, 82–9.

WELLMAN, C. & WHERRY, W. B. (1910). Some new internal parasites of the California ground squirrel. (*Otospermophilus beecheyi*). *J. Parasit.* **3**, 417–22.

WHARTON, G. W. (1938). Acarina of Yucatan Caves. *Publ. Carnegie Instn*, **491**, 137–52.

WHARTON, G. W. (1941). Acarina collected on the presidential cruise of 1938. *Smithson. misc. Coll.* **99**, no. 12, 1–8.

WILLMANN, C. (1936). *Steatonyssus spinosus*, eine neue Milbe von *Solenodon paradoxus* Brdt. *Zool. Anz.* **114**, 5/6, 152–7.

WOMERSLEY, H. (1937). Studies in Australian Acarina. Laelaptidae. *Parasitology*, **29**, 530–8.

WOMERSLEY, H. (1941*a*). Notes on the Cheyletidae (Acarina: Trombidoidea) of Australia and New Zealand, with descriptions of new species. *Rec. S. Aust. Mus.* **7**, 51–64.

WOMERSLEY, H. (1941*b*). New species of *Geckobia* (Acarina: Pterygosomidae) from Australia and New Zealand. *Trans. Roy. Soc. S. Aust.* **65**, 323–8.

YAMADA, S. (1930). Observations on a house-infesting mite (*Liponyssus nagayoi* n.sp.) which attacks human beings. *Zool. Mag., Tokyo*, **43**, 237–49.

ZURN, F. A. (1874). *Die Schmarotzer auf und in dem Korper unserer Haussaugetier sowie die durch erstere veranlassten Krankheiten, deren Behandlung und Verhutung*. 2v. Weimar.

(*MS. received for publication* 17. v. 1949.—Ed.)

INDEX OF AUTHORS

BARTGIS, IDA LOUISE. *See* REES, CHARLES W.

BAYLIS, H. A. A review of the species of *Dinobothrium* (Cestoda), with a description of a new species. (With 9 Figures in the Text), 96

BIRKETT, BETTY. *See* BISHOP, ANN

BISHOP, ANN and BIRKETT, BETTY. Some observations upon prolonged treatment of *Plasmodium gallinaceum* infection in chicks with antimalarial drugs, 149

BISHOP, ANN and MCCONNACHIE, ELSPETH W. The stability of paludrine-resistance in *Plasmodium gallinaceum* in the absence of the drug, 159

BISHOP, ANN and MCCONNACHIE, ELSPETH W. Sulphadiazine-resistance in *Plasmodium gallinaceum* and its relation to other antimalarial compounds, 163

BISHOP, ANN and MCCONNACHIE, ELSPETH W. Cross-resistance between sulphanilamide and paludrine (proguanil) in a strain of *Plasmodium gallinaceum* resistant to sulphanilamide, 175

CRAGG, J. B. The reactions of *Lucilia sericata* (Mg.) to various substances placed on sheep, 179

CRAGG, J. B. and THURSTON, BERYL A. The reactions of blowflies to organic sulphur compounds and other materials used in traps, 187

CROWCROFT, PETER. Note on *Lintonium vibex* (Linton, 1899) (Digenea—Trematoda). (With 9 Figures in the Text), 316

DAVIDOVICI, S. *See* MER, G. G.

FELDMAN-MUHSAM, B. On some abnormalities in *Hyalomma savignyi*. (With 7 Figures in the Text), 93

FINLAYSON, L. H. Mortality of *Laemophloeus* (Coleoptera, Cucujidae) infected with *Mattesia dispora* Naville (Protozoa, Schizogregarinaria). (With 4 Figures in the Text), 261

FULLER, H. S. *See* PHILIP, CORNELIUS B.

GARNHAM, P. C. C. Blood parasites of East African vertebrates, with a brief description of exo-erythrocytic schizogony in *Plasmodium pitmani*. (With Plates VII and VIII), 328

GIBBS, ALFRED J. *Crithidia familiaris* n.sp. in *Cenaeus carnifex* Fabr. (Hemiptera). (With 15 Figures in the Text), 322

GNANAMUTHU, C. P. Two dichelesthiid copepods from Madras fish. (With 33 Figures in the Text), 276

HAFEZ, M. On the behaviour and sensory physiology of the house-fly larva, *Musca domestica* L. I. Feeding stage. (With 28 Figures in the Text), 215

HAMILTON, A. G. The occurrence and morphology of *Coenurus serialis* in rabbits. (With Plates I–IV, containing Figs. 1–11), 46

JONES, B. M. A method for studying the distribution and bionomics of trombiculid mites (Acarina: Trombidiidae). (With 7 Figures in the Text), 1

JONES, B. M. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. (With 10 Figures in the Text), 247

KHALIFA, A. Spermatophore production and egg-laying behaviour in *Rhodnius prolixus* Stal. (Hemiptera; Reduviidae). (With 3 Figures in the Text), 283

KIRK, R. Experimental differentiation of *Leishmania*, 58

KIRTISINGHE, P. Parasitic copepods of fish from Ceylon. III. (With 60 Figures in the Text), 77

MACAN, T. T. Malaria survey of the Arakan region of Bengal and Burma. (With 2 Figures in the Text), 290

MCCONNACHIE, ELSPETH W. *See* BISHOP, ANN

MER, G. G. and DAVIDOVICI, S. A method for estimating the toxic effect of contact insecticides on mosquitoes and house-flies, 87

MILNE, A. The ecology of the sheep tick, *Ixodes ricinus* L. Microhabitat economy of the adult tick. (With 1 Figure in the Text), 14

MILNE, A. The ecology of the sheep tick, *Ixodes ricinus* L. Spatial distribution, 35

NEAL, R. A. An experimental study of *Entamoeba muris* (Grassi, 1879); its morphology, affinities and host-parasite relationship. (With 23 Figures in the Text), 343

PHILIP, CORNELIUS B. and FULLER, H. S. The harvest mites ('Akidani') of Japan and the Far East and their relationship to the *Autumnalis* group of trombiculid mites. (With 3 Plates and 3 Figures in the Text), 50

RADFORD, CHARLES D. The mites (Acarina) parasitic on mammals, birds and reptiles, 366

REARDON, LUCY V. *See* REES, CHARLES W.

REES, CHARLES W., REARDON, LUCY V. and BARTGIS, IDA LOUISE. The excystation of *Entamoeba histolytica* without bacteria in microcultures. (With 1 Figure in the Text), 338

REES, GWENDOLEN. Observations on the vertical migrations of the third-stage larva of *Haemonchus contortus* (Rud.) on experimental plots of *Lolium perenne* S 24, in relation to meteorological and micrometeorological factors. (With 3 Figures in the Text), 127

REES, GWENDOLEN. The plerocercoid larva of *Grillotia heptanchi* (Vaullegeard). (With 18 Figures in the Text), 265

RICHARDS, W. S. The variation of the British harvest mite (Trombiculidae, Acarina). (With 23 Figures in the Text), 105

RICHARDS, W. S. The distribution and biology of the harvest mite in Great Britain (Trombiculidae, Acarina). (With 3 Figures in the Text), 118

STAMMERS, F. M. G. Observations on the behaviour of land-leeches (genus *Haemadipsa*). (With 1 Figure in the Text), 237

TETLEY, J. H. The differentiation of the eggs of *Haemonchus contortus* and *Ostertagia* species of the sheep and a note on the relative generic egg-laying rates. (With 1 Figure in the Text), 273

THOMAS, H. T. Abnormal material bearing on the development of the terminalia of male calypterate cyclorrhaphous Diptera. (With Plate VI and 7 Figures in the Text), 304

THURSTON, BERYL A. *See* CRAGG, J. B.

TURK, F. A. A new species of parasitic mite, *Cnemidocoptes jamaicensis*, a causative agent of scaly leg in *Turdus aurantiacus*. (With 6 Figures in the Text), 60

TURK, F. A. Studies of acari. VI. Parasitic mites from mammalian hosts obtained in Ceylon. (With 15 Figures in the Text), 63

TYSSUL JONES, T. W. Anopheline larvae as intermediate hosts for larval trematodes. (With 2 Figures in the Text), 144

WILSON, S. G. An experimental study of East Coast fever in Uganda. I. A study of the type of East Coast fever reactions produced when the number of infected ticks is controlled. (With 24 Figures in the Text), 195

WILSON, S. G. An experimental study of East Coast fever in Uganda. II. The durability of immunity in East Coast fever. (With 5 Figures in the Text), 210

ZUMPT, F. Notes on parasitic mites. I. Some remarks on the family Laelaptidae (*sensu* Vitzthum 1943) with descriptions of three new species from African rodents. (With 4 Figures in the Text), 298

INDEX OF SUBJECTS

Acari, studies of, VI. Parasitic mites from mammalian hosts obtained in Ceylon, 63
Anopheline larvae as intermediate hosts for larval trematodes, 144

Blood parasites of East African vertebrates, with a brief description of exo-erythrocytic schizogony in *Plasmodium pitmani*, 328
Blowflies, the reactions of, to organic sulphur compounds and other materials used in traps, 187

Cnemidocoptes jamaicensis, a new species of parasitic mite, a causative agent of scaly leg in *Turdus aurantiacus*, 60
Coenurus serialis, the occurrence and morphology of, in rabbits, 46
Contact insecticides, a method for estimating the toxic effect of, on mosquitoes and house-flies, 87
Copepods, parasitic, of fish in Ceylon, 77
Copepods, two dichelesthiid, from Madras fish, 276
Crithidia familiaris n.sp. in *Cenaeus carnifex* Fabr. (Hemiptera), 322
Cross-resistance between sulphanilamide and paludrine (proguanil) in a strain of *Plasmodium gallinaceum* resistant to sulphanilamide, 175

Dinobothrium (Cestoda), a review of the species of, with a description of a new species, 96

East Coast fever in Uganda, an experimental study of,
I. A study of the type of East Coast fever reactions produced when the number of infected ticks is controlled, 195
II. The durability of immunity in East Coast fever, 210
Ecology of the sheep tick, *Ixodes ricinus* L. Microhabitat economy of the adult tick, 14
Ecology of the sheep tick *Ixodes ricinus* L. Spatial distribution, 35
Entamoeba histolytica, the excystation of, without bacteria in microcultures, 338
Entamoeba muris (Grassi, 1879); an experimental study of, its morphology, affinities and host-parasite relationship, 343
Experimental differentiation of *Leishmania*, 58

Grillotia heptanchi (Vaullegeard), the plerocercoid larva of, 265

Haemonchus contortus and *Ostertagia* species of the sheep, the differentiation of the eggs of, and a note on the relative generic egg-laying rates, 273
Haemonchus contortus (Rud.), observations on the vertical migrations of the third-stage larva of, on experimental plots of *Lolium perenne* S 24, in relation to meteorological and micrometeorological factors, 127
Harvest mites ('Akidani') of Japan and the Far East and their relationship to the *Autumnalis* group of trombiculid mites, 50
Hyalomma savignyi, on some abnormalities in, 93

Ixodes ricinus L., the ecology of the sheep tick, microhabitat economy of the adult tick, 14
Ixodes ricinus L., the ecology of the sheep tick, spatial distribution, 35

Laemophloeus (Coleoptera, Cucujidae) infected with *Mattesia dispora* Naville (Protozoa, Schizogregarinaria), mortality of, 261
Land-leeches (genus *Haemadipsa*), observations on the behaviour of, 237
Leishmania, experimental differentiation of, 58
Lintonium vibex (Linton, 1899) (Digenea—Trematoda), note on, 316
Lucilia sericata (Mg.), the reactions of, to various substances placed on sheep, 179

Malaria survey of the Arakan region of Bengal and Burma, 290
Mattesia dispora Naville (Protozoa, Schizogregarinaria), mortality of *Laemophloeus* (Coleoptera, Cucujidae), infected with, 261
Mites (Acarina), the, parasitic on mammals, birds and reptiles, 366
Mites, parasitic, notes on, I, some remarks on the family Laelaptidae (*sensu* Vitzthum 1943) with descriptions of three new species from African rodents, 298
Musca domestica L., on the behaviour and sensory physiology of the house-fly larva, I, feeding stage, 215

Occurrence and morphology of *Coenurus serialis* in rabbits, 46

Parasitic mites, notes on, I, some remarks on the family Laelaptidae (*sensu* Vitzthum 1943) with descriptions of three new species from African rodents, 298
Parasitic mites from mammalian hosts obtained in Ceylon, 63
Plasmodium gallinaceum, cross-resistance between sulphanilamide and paludrine (proguanil) in a strain of, resistant to sulphanilamide, 175
Plasmodium gallinaceum infection in chicks, some observations upon prolonged treatment of, with antimalarial drugs, 149
Plasmodium gallinaceum, stability of paludrine-resistance in, in the absence of the drug, 159
Plasmodium gallinaceum, sulphadiazine-resistance in, and its relations to other antimalarial compounds, 163

Rhodnius prolixus Stal. (Hemiptera; Reduviidae), spermatophore production and egg-laying behaviour in, 283

Spermatophore production and egg-laying behaviour in *Rhodnius prolixus* Stal. (Hemiptera; Reduviidae), 283

Stability of paludrine-resistance in *Plasmodium gallinaceum* in the absence of the drug, 159
Sulphadiazine-resistance in *Plasmodium gallinaceum* and its relation to other antimalarial compounds, 163

Terminalia of male calypterate cyclorrhaphous Diptera, abnormal material bearing on the development of the, 304
Trematodes, anopheline larvae as intermediate hosts for larval, 144
Trombicula autumnalis Shaw, the harvest mite, the penetration of the host tissue by, 247
Trombiculidae, Acarina, the distribution and biology of the harvest mite in Great Britain, 118
Trombiculidae, Acarina, the variation of the British harvest mite, 105
Trombiculid mites (Acarina: Trombidiidae), a method for studying the distribution and bionomics of, 1
Trombiculid mites, harvest mites ('Akidani') of Japan and the Far East and their relationship to the *Autumnalis* group of, 50
Turdus aurantiacus, Cnemidocoptes jamaicensis, a new species of parasitic mite, a causative agent of scaly leg in, 60

Publications issued by

COMMONWEALTH INSTITUTE OF ENTOMOLOGY

41 Queen's Gate, London, S.W. 7

BULLETIN OF ENTOMOLOGICAL RESEARCH

Published quarterly and containing original articles on Economic Entomology. *Issued Post Free.*

Annual Subscription (payable in advance). Vol. 41 (1950) et sqq. 60*s.* 0*d.*

Prices of back parts and volumes on application.

REVIEW OF APPLIED ENTOMOLOGY

Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series:

Series "A" dealing with insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin.

Series "B" dealing with insects, ticks, etc. conveying disease or otherwise injurious to man and animals. *Issued Post Free.*

	Series "A"	*Series "B"*
Annual Subscription (payable in advance)	40*s.* 0*d.*	20*s.* 0*d.*

Prices of back parts and volumes on application.

ZOOLOGICAL RECORD, part INSECTA

Published annually about October each year and containing as complete a record as possible of the literature of the previous year, chiefly from the systematic standpoint.

Annual Subscription (including postage) vol. 84 et sqq. 20*s.* 6*d.*

Prices of back volumes on application.

TICKS

A MONOGRAPH OF THE IXODOIDAE

By

GEORGE H. F. NUTTALL, CECIL WARBURTON
W. F. COOPER and L. E. ROBINSON

Part I. Argasidae. 8*s.* 6*d.* *net*

Part II. Ixodidae. 16*s.* *net*

Bibliography I. 8*s.* 6*d.* *net*

Bibliography II. 7*s.* 6*d.* *net*

Fully illustrated by numerous text-figures and plates.
Each part is complete in itself.

CAMBRIDGE UNIVERSITY PRESS

CONTENTS

(*All rights reserved*)

PAGE

WILSON, S. G. An experimental study of East Coast fever in Uganda. I. A study of the type of East Coast fever reactions produced when the number of infected ticks is controlled. (With 24 Figures in the Text) 195

WILSON, S. G. An experimental study of East Coast fever in Uganda. II. The durability of immunity in East Coast fever. (With 5 Figures in the Text) . . . 210

HAFEZ, M. On the behaviour and sensory physiology of the house-fly larva, *Musca domestica* L. I. Feeding stage. (With 28 Figures in the Text) 215

STAMMERS, F. M. G. Observations on the behaviour of land-leeches (genus *Haemadipsa*) (With 1 Figure in the Text) 237

JONES, B. M. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. (With 10 Figures in the Text) 247

FINLAYSON, L. H. Mortality of *Laemophloeus* (Coleoptera, Cucujidae) infected with *Mattesia dispora* Naville (Protozoa, Schizogregarinaria). (With 4 Figures in the Text) 261

REES, GWENDOLEN. The plerocercoid larva of *Grillotia heptanchi* (Vaullegeard). (With 18 Figures in the Text) 265

TETLEY, J. H. The differentiation of the eggs of *Haemonchus contortus* and *Ostertagia* species of the sheep and a note on the relative generic egg-laying rates. (With 1 Figure in the Text) 273

GNANAMUTHU, C. P. Two dichelesthiid copepods from Madras fish. (With 33 Figures in the Text) 276

KHALIFA, A. Spermatophore production and egg-laying behaviour in *Rhodnius prolixus* Stal. (Hemiptera; Reduviidae). (With 3 Figures in the Text) . . 283

MACAN, T. T. Malaria survey of the Arakan region of Bengal and Burma. (With 2 Figures in the Text) 290

ZUMPT, F. Notes on parasitic mites. I. Some remarks on the family Laelaptidae (*sensu* Vitzthum 1943) with descriptions of three new species from African rodents. (With 4 Figures in the Text) 298

THOMAS, H. T. Abnormal material bearing on the development of the terminalia of male calypterate cyclorrhaphous Diptera. (With Plate VI and 7 Figures in the Text) 304

CROWCROFT, PETER. Note on *Lintonium vibex* (Linton, 1899) (Digenea—Trematoda). (With 9 Figures in the Text) 316

GIBBS, ALFRED J. *Crithidia familiaris* n.sp. in *Cenaeus carnifex* Fabr. (Hemiptera). (With 15 Figures in the Text) 322

GARNHAM, P. C. C. Blood parasites of East African vertebrates, with a brief description of exo-erythrocytic schizogony in *Plasmodium pitmani*. (With Plates VII and VIII) 328

REES, CHARLES W., REARDON, LUCY V. and BARTGIS, IDA LOUISE. The excystation of *Entamoeba histolytica* without bacteria in microcultures. (With 1 Figure in the Text) 338

NEAL, R. A. An experimental study of *Entamoeba muris* (Grassi, 1879); its morphology, affinities and host-parasite relationship. (With 23 Figures in the Text) . . . 343

RADFORD, CHARLES D. The mites (Acarina) parasitic on mammals, birds and reptiles 366

Index of Authors 395

Index of Subjects 397

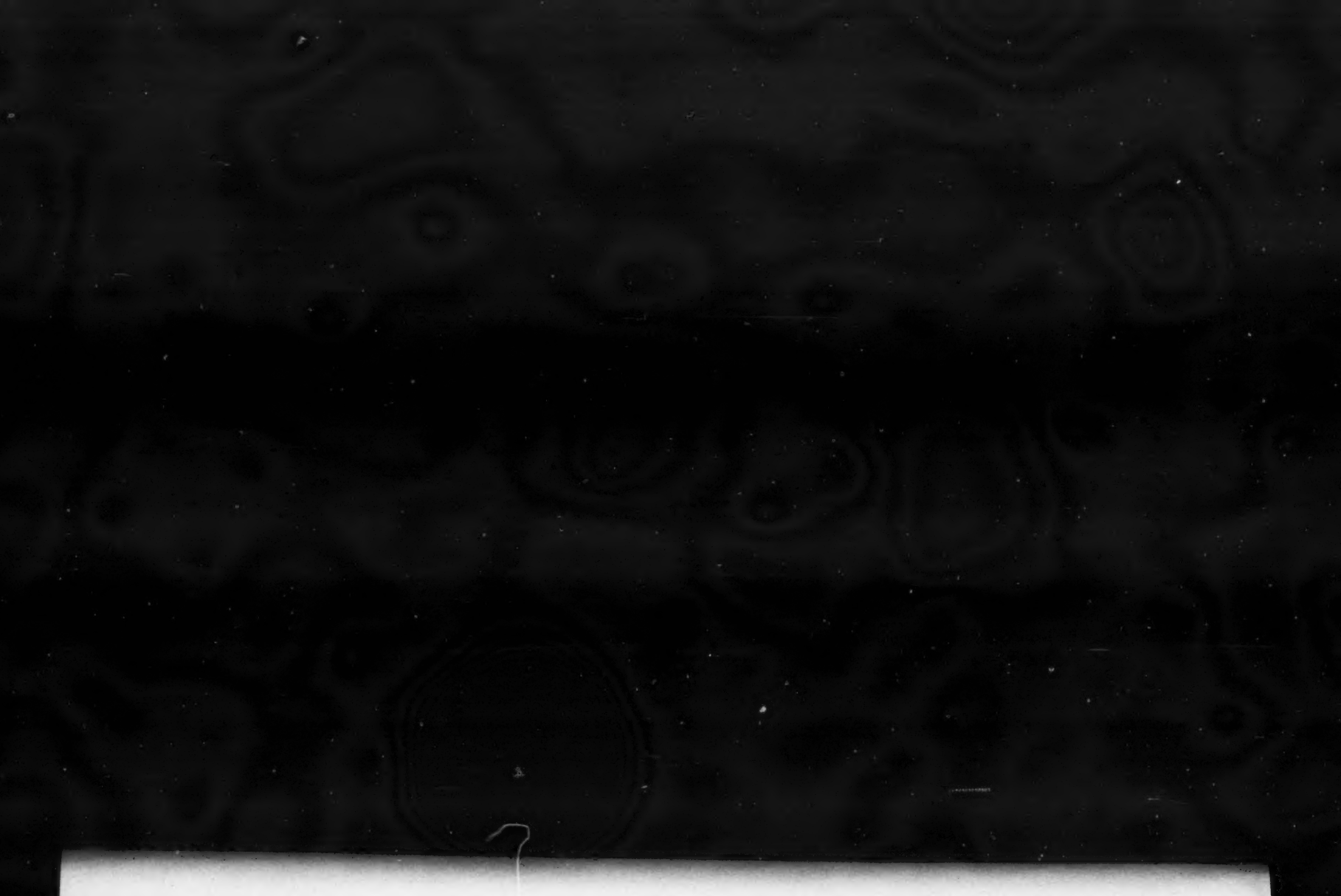
PARASITOLOGY is published three or four times a year. The numbers afterwards are issued in volumes each containing four numbers.

Papers for publication should be sent to Professor D. KEILIN, Molteno Institute, Downing Street, Cambridge. Other communications should be addressed to the University Press, Cambridge.

Papers forwarded to the Editors for publication are understood to be offered to *PARASITOLOGY* alone, unless the contrary is stated.

Contributors receive fifty copies of their papers free. Additional copies, not exceeding one hundred (except in special cases), may be had at cost price: these should be ordered when the final proof is returned.

The subscription price is £2. 15*s*. per volume (post-free), payable in advance; single numbers 18*s*. 6*d*. net (double number 37*s*. net). Subscriptions may be sent to any Bookseller, or to The Cambridge University Press, Bentley House, 200 Euston Road, N.W. 1.

Enquiries from the U.S.A. should be addressed to the American Branch of the Press at 51 Madison Avenue, New York, 10.

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)